PB80-182843

Toxicity of Metals to Marine Phytoplankton Cultures

Texas A and M Univ. at Galveston

Prepared for

Environmental Research Lab.
Narragansett, RI

Feb 80

U.S. Department of Commerce
National Technical Information Service
NTIS

United States
Environmental Protection
Agency

Environmental Research
Laboratory
Narragansett RI 02882

EPA-600/3-80-025
February 1980

PB80-182843

Research and Development

EPA

Toxicity of Metals to Marine Phytoplankton Cultures

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial, and atmospheric environments.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.
EPA-600/3-80-025

2.

3. RECIPIENT'S ACCESSION NO.
PB 80 182843

4. TITLE AND SUBTITLE
TOXICITY OF METALS TO MARINE PHYTOPLANKTON CULTURES

5. REPORT DATE
February 1980 issuing date

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
William B. Wilson and Larry R. Freeberg

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Texas A&M University at Galveston
Galveston, Texas 77550

10. PROGRAM ELEMENT NO.
1BA819

11. CONTRACT/GRANT NO.
R801511

12. SPONSORING AGENCY NAME AND ADDRESS
Environmental Research Laboratory - Narragansett, RI
Office of Research and Development
U.S. Environmental Protection Agency
Narragansett, Rhode Island 02882

13. TYPE OF REPORT AND PERIOD COVERED
Final

14. SPONSORING AGENCY CODE
EPA/600/05

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The objectives of this program were to evaluate the toxicity of nine metals to cultures of four species of marine phytoplankton. The relationships of acute, instantaneous and chronic toxicity were evaluated using growth rates in continuous-flow culture systems. The latter methods employed both the chemostat and the turbidostat techniques. The instantaneous procedure measures short-term changes of metabolic activity indicated by 14-C uptake that result from metal addition within a relatively short time period after cultures are exposed to metal additions. Four levels were determined for the acute toxicity of metals to each organism. The use of fluorometric measurements of relative chlorophyll-a of actively growing cultures was a fast, accurate assay method that facilitated the Minimum Toxicity Level calculation, increased the sensitivity of the method, and reduced variability. Refinement should result in this method being more useful for giving more uniform assay results.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS				b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
			selenium		
Marine Biology	0801	0603	Copper	Continuous culture	06F
Phytoplankton	0603		Mercury	G. Splendens	06T
Salinity	0704		Silver	I. Galbana	08A
Toxicity	0620		Nickel	T. pseudonana	
cobalt			cadmium	G. Halli	
			lead		
			barium		

18. DISTRIBUTION STATEMENT
RELEASE TO PUBLIC

19. SECURITY CLASS *(This Report)*
UNCLASSIFIED

20. SECURITY CLASS *(This page)*
UNCLASSIFIED

21. NO. OF PAGES
110

22. PRICE

EPA Form 2220-1 (9-73)

☆ U.S. GOVERNMENT PRINTING OFFICE: 1980-657-146/5572

EPA-600/3-80-025
February 1980

TOXICITY OF METALS TO MARINE PHYTOPLANKTON CULTURES

by

William B. Wilson and Larry R. Freeberg
Texas A&M University at Galveston
Galveston, Texas 77550

Grant No. R801511

Project Officer

C. S. Hegre
Environmental Research Laboratory
Narragansett, Rhode Island 02882

This study was conducted
in cooperation with
Marine Research Laboratory
Texas A&M University at Galveston
Galveston, Texas 77550

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
NARRAGANSETT, RHODE ISLAND 02882

DISCLAIMER

This report has been reviewed by the Environmental Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

The Environmental Research Laboratory of the U.S. Environmental Protection Agency is located on the shore of Narragansett Bay, Rhode Island. In order to assure the protection of marine resources, the laboratory is charged with providing a scientifically sound basis for Agency decisions on the environmental safety of various uses of marine systems. To a great extent, this requires research on the tolerance of marine organisms and their life stages as well as of ecosystems to many forms of pollution stress. In addition, a knowledge of pollutant transport and fate is needed.

This report describes a five-year study of the relationship between growth conditions of marine phytoplankton and the toxicity of metals to them. It illustrates that toxicity is governed not only by the composition of seawater but also by the duration of exposure. An approach to toxicity testing which minimizes the uncertanties caused by these variations has been devised.

Tudor Davies
Director, ERL-Narragansett

PREFACE

As more knowledge is developed regarding various features of the marine environment, the role of the trace metals and their activities emerge as a more and more prominent feature. Not only do they act as toxicants, but also as stimulants. As more of their interactions with other metals and trace amounts of other substances are recognized, the apparent importance of metals activity in marine productivity becomes more prominent. The source, availability and chemical form of metals become more important and the activity of chemical and physical conditions demands consideration in the environment, especially in regard to productivity. Also, as more data become available, the rewards of sophisticated research procedures become obvious and the values of long-used techniques and methods show the merit they deserve as standards.

Perhaps this work will emerge as a more sensitive assay procedure that will reduce the work per analysis in its simplest form and that will increase the sensitivity of phytoplankton bioassays, so that it can be employed as a standard technique.

The turbidostat and chemostat procedures show sufficient capabilities to merit additional efforts to perfect these procedures for regular use. Although these assays were time-consuming and were subject to operational failures, the possibility of detecting long-term effects of toxicants on organisms merits additional evaluation. Results indicated that population reductions not detectable by other means may be discerned in a continuous-flow system.

ABSTRACT

The objectives of this program were to evaluate the toxicity of nine metals to cultures of four species of marine phytoplankton. The relationships of acute, instantaneous and chronic toxicity were evaluated using growth rates in continuous-flow culture systems. The latter methods employed both the chemostat and the turbidostat techniques. The instantaneous procedure measures short-term changes of metabolic activity indicated by 14-C uptake that result from metal addition within a relatively short time period after cultures are exposed to metal additions. Four levels were determined for the acute toxicity of metals to each organism. The use of fluorometric measurements of relative chlorophyll *a* of actively growing cultures was a fast, accurate assay method that facilitated the Minimum Toxicity Level calculation, increased the sensitivity of the method, and reduced variability. Refinement should result in this method being more useful for giving more uniform assay results. Also, the procedures are necessary for this method to succeed.

The medium composition in which they live was apparently the main factor that protected phytoplankton from the toxic effects of metallic ions. Sea water that is "more pure", i.e., contains none or low amounts of dissolved organic substances not usually found in sea water, probably offers less protection to the phytoplankton populations than the so-called polluted water. On the other hand, polluted waters impose more variable conditions which usually result in the survival of fewer species. That is, the sea water components that protect estuarine organisms from metals toxicity may limit the growth of many species directly.

Results indicated that the nine metals tested could be grouped based on their acute toxicity. The toxicity levels of the groups differed by approximately one order of magnitude from 0.05 mg/L, 0.5 mg/L and 5.0 mg/L. Cu, Hg and Ag comprise the most toxic group and Mo, V and Ni were the least toxic group. This group arrangement did not apparently apply to conditions of continuous-flow studies. At least the differences in toxicity between members of the groups were not an order of magnitude different in concentration.

Differences in salinity had more effect on metals toxicity than did differences in temperature. The use of salinity levels that were near the lower salinity tolerance levels of an assay organism may have had a major influence on this result. There was no significant effect within the range of temperatures tested, on the toxicity of metals to the cultures. Metals were more toxic in low salinity medium than in high salinity medium or in the combined conditions of low salinity and high temperature. High salinity (>30 ppt) reduced the Cu and Hg toxicity to *G. splendens*. Also, there was

an indirect effect of salinity on the toxicity of Cu and Ag to *G. splendens.* The major effects of salinity on toxicity was in medium that had salinity levels similar to the low level tolerated by the organism. For example, *T. pseudonana* cultures were more susceptible to metals in low salinity medium (3-7 ppt) than in high salinity medium (28 ppt). Also, nine metals were less toxic in medium with the maximum salinity levels employed (28 ppt) than in media with lower salinity levels.

The four organisms employed in the toxicity assays were not equally sensitive to the nine metals tested. The two dinoflagellates were more susceptible to metals toxicity. *G. splendens* was possibly more sensitive collectively than *G. halli* and the metals had the least effect on *I. galbana*. Also, the toxicity levels were different between metals. For example, the Hg LMTL varied from 0.005 mg Hg/L for *G. splendens* to 0.1 mg/L for *I. galbana*. The basic analysis employed in the toxicity evaluations was similar to that employed to assay the "natural" chelation level of sea water (Davey 1973; as described by Hannan and Palouillet, 1972, for fresh water pollutants and algae growth). A good feature of this assay is that each test portion can be measured repeatedly without opening the container portion. The most toxic metal group (Hg^{++}, Cu and Ag) was much more toxic than the least toxic group (Ni, V and Mo). The toxic levels of Ni, V, and Mo were 6 to 10 times less toxic than Hg, Cu and Ag. Individually, Ag was the most toxic metal tested.

Differences in the low (LMTL) and high (HMTL) results indicated a 10-fold or more range between the two levels and these differences increased as the toxicity of the metals decreased. For example, the LMTL of Cu to *G. splendens* was one-tenth the HMTL of Cu but the LMTL and HMTL of vanadium was one-twentieth.

Results of residual, instantaneous and chronic toxicity experiments indicated that these four organisms could be subjected to high concentrations of the nine metals for periods of 24 to 144 hours and subsequently grow after being transferred to fresh medium. Some reports indicated that phytoplankton "take-up" metals rapidly. Thus, the lack of continued mortality of cultures after transfer to fresh medium must indicate that some short-term growth effects are not permanent. Indeed, some of the effects observed could represent that part of culture population development commonly referred to as "lag phase." These effects were exemplified by the results of the instantaneous and residual toxicity results. Cultures were tolerant of relatively high metals concentrations and had a high degree of recovery from toxic levels. The order of toxicity (i.e., most toxic to least toxic metals) in chemostat tests was similar to that of the acute toxicity results.

Instantaneous toxicity experiments showed that the metals produced a rapid, short-term reduction of ^{14}C uptake, but the effects were transient and recovery occurred, in some cases, within 1 hour. Low amounts of some metals (as low as 10 μg Cu/L) reduced both the growth rate and ^{14}C uptake of *I. galbana*, but concentrations as high as 1250 μg Cu/L did not stop cell division. The growth of *I. galbana* in instantaneous experiments and turbidostat cultures was stimulated by Hg additions of 10 μg Hg/L or less and by 11.2 μg Cr/L.

The optimum temperature for the growth of *I. galbana* in batch culture was from 20 to 25C (Kain and Fogg, 1958). The growth was less at temperatures below 15C and above 25C. The optimum temperatures for growth in continuous cultures was from 17 to 26C (Freeberg, 1971). Growth was significantly less at 17C or below and above 26C. Data on the combined effects of temperature and salinity on growth often indicated that the optimum temperature level was between 16 and 24C, but not as high as 28 or as low as 12C.

Turbidostat results were similar to those from the chemostat, but differences did occur. Strickland (1965) hypothesized that there was possibly a temperature range of 10C within which the division time of an algal cell was essentially constant. On the other hand, Freeberg (1971) found the K_c changed with each change (3C) in temperature. The equation for the linear increase in the K_c of *I. galbana* from 14 to 26C was $y = 0.0645(x)-0.281$ (Freeberg, 1971). In this study it was $y = 0.065(x)-0.295$ with a correlation coefficient of 0.99.

Cells in a nutrient-rich culture may become light-limited by way of mutual shading associated with high population levels and growth may be limited in a manner similar to cells in a nutrient-limited culture. The K_c of *I. galbana* did not change significantly in cultures with cell concentrations from 1.0×10^5 to 1.75×10^5 cells/ml, but the K_c decreased (21%) with 2.0×10^5 cells/ml and 5 mw/cm^2 irradiation. A light-limited culture regime was indicated by results with 1.75×10^5 and 2.0×10^5 cells/ml and 5 or 10 mw/cm^2 irradiation.

The variability of K_c values may be partially attributed to the relatively low concentration of cells in the cultures. Batch cultures contained from 2 to 14×10^6 cells/ml (Kain and Fogg, 1958). Two-liter chemostats contained from 1.6 to 3.0×10^5 cells/ml (Caperon, 1968). In batch cultures the population level is continuously changing and the growth rate is integrated over a range of cell concentrations. The minimum population level (2×10^6 cells/ml) (Kain and Fogg, 1958) was an order of magnitude greater than the concentration indicated as light-limited in this study. The maximum growth rate of 0.55 was similar to the K_c of light-limited cultures in this study. Light intensity, culture volume, and culture vessel width may also control the concentration at which the cells become light-limited.

The variation in the growth of *G. halli* at 17 different combinations of light and temperature supported the previously described hypothesis of constant algal growth within wide ranges of light and temperature (Strickland, 1965). The optimum temperature for the growth of *G. halli* was from 23 to 32C, however, the K_c values changed with each 3C change from 23 to 29C. Light intensities had little effect upon the K_c of *G. halli,* yet the cell concentration was directly related to the light intensity. The K_c of *G. halli* with 10 mw/cm^2 irradiation declined significantly only after the cell number exceeded approximately 2.4×10^4 cells/ml. A 50% increase in irradiation from 10 to 15 mw/cm^2 increased the cell number 2.5 times to 6.1×10^4 cells/ml. In contrast to *I. galbana,* the Chl *a*/cell of *G. halli* was not related to the cell number. These data further supported the hypothesis that algal growth may become limited at moderate cell numbers in cultures, and algal bioassay tests may be insensitive to subtle changes due to prior suppression (light-limited) of algal growth.

The action of a heavy metal on living cells has been shown to be a two-step process. The first action site occurs at the surface boundaries of the cell. As the metal diffuses into the cell, the second action occurs intracellularly (Rothstein, 1959). The first reactions are characterized by adsorption of the metal on the cell membrane, followed by a physiological disturbance, if any, resulting from the chemical alterations of the diffusion barrier by the membrane-bound metal. These reactions are relatively rapid and are usually reversible, while the interactions within the cell, the second action, exhibit a time lag and are not essentially reversible (Rothstein, 1959).

The first actions have been demonstrated for copper and mercury with a number of phytoplankton species. Mandelli (1969) reported a rapid uptake of copper by nine species of marine phytoplankton for the first 15 to 30 minutes of exposure. Continued Cu uptake, at a reduced rate, was dependent upon cell division. Glooschenko (1969) found that dead, live non-dividing and live dividing cells of the marine diatom *Chaetoceros costatum* initially took up ^{203}Hg rapidly, while only live dividing cells in the light continued to take up Hg. The initial Hg uptake was not dependent on photosynthesis and Glooschenko suggested that, although some active uptake may occur in actively metabolizing cells, the most important process in Hg uptake by *Chaetoceros* was passive surface adsorption. The primary effect of cytoplasmic membrane bound Hg in *Chlorella* was light-independent excretion of potassium through the diffusion barrier (Kamp-Nielsen, 1971). Cell division was inhibited by the alteration in membrane permeability, yet the light processes of photosynthesis were not affected until Hg ions penetrated the cells. Lag period in cell division, indicating the first type of reaction, were extended in relation to the initial Hg concentration, yet the extended lag periods could be reduced or eliminated by complexing the Hg with the addition of citric acid. Nuzzi (1972) noted that inhibition in the growth of *Phaeodactylum tricornutum* by Hg (as phenylmercuric acetate) was reversed with the addition of monothiol compounds, e.g., glutathione. Ben-Bassat *et al* (1972) reported the lag period prior to the growth of *Chlamydomonas reinhardi* was directly related to the initial Hg concentration in the medium, and suggested that recovery to a normal growth rate was due to loss of Hg from not only the cells but also from the total culture system. He determined that mercury was reduced by 40% in the culture system (medium and cells) after 8 days whereas there was a 5% reduction in the controls system. They theorized the Hg was biologically converted to a volatile and less permeable form, which had less affinity to the cells. In other words, a single sublethal dose of Hg inhibits algal cell division, apparently via alteration in cell membrane permeability, and upon natural or induced removal of the membrane-bound Hg, normal cell division resumed.

The cytoplasmic concentration of Hg in *Isochrysis galbana* cultures was ca 10^5 times that in the medium, and intracellular uptake was controlled by membrane diffusions (Davies 1974, 1976). A greater driving concentration of Hg than initially added to the medium was required for the calculated diffusion coefficients, and Davies suggested that membrane adsorption was the mechanism that enabled such a buildup of Hg at the cell surfaces. Davies reported the specific growth rate of *I. galbana* was inversely related to the intracellular concentration of Hg. He was not able to distinguish a difference in toxicity of surface adsorbed or intracellular Hg, thus the site or mode of Hg toxicity has yet to be demonstrated. Davies (1974) reported an increase in the mean cell volume of *I. galbana* when initially exposed to Hg and suggested the enlarged cells were probably due to inhibition of cell division by the metal forming complexes with sulphur-containing compounds known to be important for cell division, e.g., methionine. Moreover, Davies (1976) was not able to determine any significant excretion of intracellular potassium by *I. galbana* or *Dunaliella tertiolecta* after exposure to Hg. He concluded that, "The primary effect of mercury upon phytoplankton was not disruption of cell membranes causing loss of intracellular cations, but the dislocation (probably intracellular) of metabolic processes governing growth and division."

Experimental data derived from continuous exposure of algal cells to steady-state concentrations of Hg in turbidostats indicated there were Type I reactions over a wide range of Hg concentrations. First, the K_c of both *I. galbana* and *G. halli* were affected when the cultures were exposed to Hg, yet intracellular metabolic parameters (^{14}C uptake and Chl *a*) were not affected until the lethal concentration was approached. Second, the Hg induced effect upon the K_c of each species, either enhancement or inhibition, was rapidly reversed and the K_c was equivalent to that of the control when the addition of Hg was discontinued. Third, metabolic functions are usually temperature-dependent, yet the K_c of *G. halli* grown in a wide range of Hg concentrates at 23 and 30C was temperature-independent. Unfortunately, analysis of membrane and intracellular Hg concentrations similar to those of Davies (1976) were not made in this study, and intracellular metabolic parameters, as reported in this study were not determined by Davies. A study employing continuous culture techniques and analysis of a variety of metabolic parameters in conjunction with isolating the cellular location of Hg concentrations would contribute to the elucidation of mercury's mode of action. Also, it is suggested that the effect of Hg upon membrane permeability and/or uptake of ions or molecules known to be important for cell division should be included in future studies, e.g., sulfur, usually taken up as SO_4, is required for cell division and the inhibition of sulfur uptake could be a passive mechanism which reduced cell division without an effect upon metabolic parameters, such as ^{14}C uptake.

I. galbana and *G. halli* were not only more sensitive to chronic exposures of Cr than to Hg, but the mode of action of Cr appeared to be different. Although a separation of Type I and Type II reactions were not discernible, internal metabolic parameters of both species were affected by Cr, and the K_c was directly related to the changes in ^{14}C uptake or Chl *a*/cell. The Chl *a*/cell of *I. galbana* was not significantly affected by Cr while the ^{14}C uptake was inversely related to the Cr concentration over the total range of Cr tested. The ^{14}C uptake of *G. halli* was not significantly affected by Cr but the Chl *a*/cell was greater than that of the control culture if the Cr concentrations were less than 12 μg/L. The Chl *a*/cell of *G. halli* was inversely related to the medium Cr concentration from approximately 7.9 μg Cr/L to the maximum concentration tested (35.6 μg Cr/L).

A model for species succession and/or community dominance by one species was illustrated not only by the difference in the sensitivity of the species tested, but also by the dissimilar patterns of response exhibited by each species exposed to Hg and Cr. For example, Hg was stimulatory, inhibitory, and lethal to the growth of *G. halli* within a range that did not significantly affect the growth of *I. galbana*.

Although some results indicated that metals were more toxic in chemostat cultures than in turbidostat cultures, the high sensitivity in the former is believed to result from the condition of chemostat populations. Physiologically a stressed population would be most likely to be more sensitive to metals than a non-stressed population. Although we have no measure of the stress that exists in a phytoplankton culture, a declining population is probably more subject to metals toxicity than a fast-growing population.

CONTENTS

Foreword iii
Preface iv
Abstract v
Figures xii
Tables xiii
Abbreviations and Symbols xvi
Acknowledgments xvii
SECTION 1. Introduction 1
SECTION 2. Acute Toxicity of Nine Metals to Marine Phytoplankton Cultures 5
Materials and Methods 5
Results 12
SECTION 3. Residual Toxicity of Nine Metals to *G. splendens, I. galbana, T. pseudonana* and *G. halli*. 19
Results 20
SECTION 4. The Effects of Temperature and Salinity on the Acute Toxicity of Metals to *G. splendens, I. galbana* and *T. pseudonana* 24
Materials and Methods 24
Results 25
SECTION 5. Instantaneous Effects of Metals on Photosynthesis Rates of *G. splendens, T. pseudonana* and *I. galbana*. 36
Materials and Methods 36
Results 37
SECTION 6. The Effects of Light Intensity, Temperature, Cell Density and Chronic Exposure of Mercury and Chromium on Two Marine Phytoplankters in Continuous Culture 38
Materials and Methods 40
Results 46
SECTION 7. The Chronic Toxicity of Mercury, Chromium and Nickel to *G. halli* and *I. galbana* (Chemostat) . . . 61
Materials and Methods 61
Results 63
SECTION 8. References 81

FIGURES

Number Page

1 Variability of Cu toxicity to *G. splendens* (values in mg/L) . . . 9

2 The relationship of MTL, NTL, NGL and TML to the toxicity of Cu to *G. splendens* (results from 11 experiments). 10

3 Diagrammatic sketch of the basic turbodistat design 41

4 Diagrammatic sketch of the basic chemostat design 62

5 Response of *G. halli* culture to Hg concentration of 50 mμg/L (one experiment). 65

6 Response of *G. halli* culture to Ni concentration of 0.2 μg/L (one experiment). 66

7 Response of *G. halli* culture to Hg concentration of 2.0 mμg/L (one experiment). 67

8 Response of *G. halli* culture to Ni concentration of 0.15 μg/L (one experiment). 68

TABLES

Number Page

1 Composition of Chelate-Free Artificial Seawater Medium which Supported the Best Growth of *I. galbana*, *T. pseudonana*, *C. nana*, *G. splendens* and *G. halli* 6

2 Acute Toxicity of Nine Metals to Cultures of Four Marine Phytoplankton Organisms (values in mg/L) 13

3 The Low Minimum Toxicity Level (LMTL-1), Low No Growth Level (LNGL-2) and Low Total Mortality Level (LTML-3) of Nine Metals to Four Phytoplankton Cultures (values in mg/L) 15

4 Group List of Nine Metals Based on Their Gravimetric (mg/L) and Molecular (μg at/l) Weights of Combined LMTL and LNGL Acute Toxicity Values to *G. halli*, *G. splendens*, *I. galbana* and *T. pseudonana* 17

5 High Minimum Toxicity Level (HMTL-1), High No Growth Level (HNGL-2) and High Total Mortality Level (HTML-3) of Nine Metals to Four Marine Phytoplankton Cultures 18

6 Residual Toxicity (A) of Nine Metals to *I. galbana*, *T. pseudonana* and *G. splendens* (values in mg/L) 21

7 Residual Toxicity (A) of Nine Metals to *G. halli* After Exposures of 1, 24, 48, 72 and 144 Hours 22

8 Effects of Temperature and Salinity on the Growth Rates (K) of *G. splendens*, *I. galbana* and *T. pseudonana* 26

9 The Effects of Temperature on the Toxicity of Metals to *G. splendens* (values in mg/L) 28

10 Effects of Salinity on the Toxicity of Metals to *G. splendens* and *I. galbana* (values in mg/L) 29

11 Combined Effects of Temperature and Salinity on the Toxicity of Metals to *G. splendens* (values in mg/L) 31

TABLES (continued)

Number Page

12 Effects of Temperature-Salinity Combinations on the Toxicity of Zn, Hg, Ag and Cu to *I. galbana* 32

13 Combined Effects of Temperature and Salinity on the Toxicity of Metals to *T. pseudonana* (clone 3H) (values in mg/L) 33

14 Specific Growth Rate, Flux, Chl *a*/Cell and ^{14}C Uptake for Various Population Levels of *I. galbana* Grown at 23C and with 5 and 10 mw/cm^2 Irradiation. 47

15 Specific Growth Rate, Chl *a*/Cell, and ^{14}C Uptake of *I. galbana* Exposed to Various Light-Temperature Combinations 48

16 Specific Growth Rate, Flux, Chl *a*/Cell and ^{14}C Uptake for Various Population Levels of *G. halli* Grown at 23C and with 5, 10 mw/cm^2 Irradiation 50

17 Specific Growth Rate, Chl *a*/Cell and ^{14}C Uptake of *G. halli* Exposed to Various Temperature-Irradiation Combinations. 52

18 Specific Growth Rate, Chl *a*/Cell, and ^{14}C Uptake of *I. galbana* Grown at 23C with 10 mw/cm^2 Irradiation and Continuous Exposure to Various Concentrations of $HgCl_2$ 55

19 Specific Growth Rate, Chl *a*/Cell, and ^{14}C Uptake of *G. halli* Grown at 30C with 10 mw/cm^2 Irradiation and Continuous Exposure to Various Concentrations of Hg^{++} 56

20 Specific Growth Rate, Chl *a*/Cell, and ^{14}C Uptake of *G. halli* Grown at 23C with 10 mw/cm^2 Irradiation and Continuous Exposure to Various Concentrations of Hg^{++} 58

21 Specific Growth Rate, Chl *a*/Cell, and ^{14}C Uptake of *I. galbana* at 23C with 10 mw/cm^2 Irradiation and Continuous Exposure to Various Concentrations of $CrCl_3$ 59

22 Specific Growth Rate, Chl *a*/Cell and ^{14}C Uptake of *G. halli* at 30C with 10 mw/cm^2 Irradiation and Continuous Exposure to Various Concentrations of $CrCl_3$ 60

TABLES (continued)

Number Page

23 Chronic Toxicity of Mercury to *G. halli* in a Chemostat 70

24 Chronic Toxicity of Chromium to *G. halli* in a Chemostat 72

25 Chronic Toxicity of Nickel to *G. halli* in a Chemostat 74

26 Chronic Toxicity of Mercury to *I. galbana* in a Chemostat 76

27 Chronic Toxicity of Chromium to *I. galbana* in a Chemostat . . . 77

28 Chronic Toxicity of Nickel to *I. galbana* in a Chemostat 79

LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS AND SYMBOLS

Cu	-- copper
Zn	-- zinc
Ag	-- silver
Co	-- cobalt
Pb	-- lead
Ni	-- nickel
Se	-- selenium
Ba	-- barium
Cd	-- cadmium
mg.	-- milligram
Fe	-- iron
Mo	-- molybdenum
V	-- vanadium
Cr	-- chromium
20C	-- twenty degrees centigrade
ppt	-- parts per thousand
L.	-- liter
ml.	-- milliliter
p.s.i.	-- pounds per square inch
B	-- boron
Mn	-- manganese
Ti	-- titanium
Si	-- silica
Zr	-- zirconium
NH_4Cl	-- ammonium chloride
$MgCl_2$	-- magnesium chloride
$NaACO_3$	-- sodium bicarbonate
pH	-- symbol for hydrogen ion concentration
EDTA	-- ethylene diamine tetra-acetic acid
chl *a*	-- chlorophyll *a*
in vitro	-- in glass, transfers were made without transferring the culture or any portion thereof from the glass container
%	-- percent or percentage
<	-- less than
>	-- more than
µg	-- microgram
Carbon-14 or ^{14}C	-- carbon-14 isotope
$\bar{X}$	-- mean numerical value
No	-- number
σ	-- standard deviation
var	-- variation

ACKNOWLEDGMENTS

The cooperation and suggestions of personnel of the National Marine Water Quality Laboratory, Office of Research and Development, U.S. Environmental Protection Agency are gratefully acknowledged. Specifically the efforts and consideration of C. S. Hegre, project officer, and S. Erickson, M. Malcomb and E. Davey in the planning and evaluation of this project were most helpful and appreciated. Many problems that occurred during various aspects of this work were made less difficult by the efforts and cooperation of M. Watson, G. Marlatt and A. Aldrich.

During various stages of this project, as many as three independent investigators were concerned with different phases of the program. One principal contributor, Larry R. Freeberg, planned, conducted research and prepared reports on the material included as Section 6, pages 38-59, titled "The Effects of Light, Temperature, Cell Density and Chronic Exposure of Mercury and Chromium on Two Marine Phytoplankters in Continuous Culture." Another contributor of the work reported herein as Section 5, "Instantaneous Effect of Metals on Photosynthesis Rate of *G. splendens, T. pseudonana* and *I. galbana*" was Emory A. Sutton. Much of the work reported here was developed and conducted independently, yet in many ways, the results were similar from one section to another.

SECTION 1

INTRODUCTION

That heavy metals are toxic to aquatic organisms has probably been known to some extent since the Phoenicians used pitch and possibly copper sheathing on their ships to prevent the attachment of marine organisms (Masseille, 1933). Also, lead sheathing, sheathing of other metals and sheathing composed of mixtures of metals were used for this purpose, but Sir Humphrey Davy was possibly the first to conduct experimental studies on the prevention of the attachment of marine organisms to vessels by use of copper sheathing (Woods Hole Oceanographic Institution, 1952). Subsequently, and at present, copper, mercury and to a lesser extent other metals are employed as the active component of anti-fouling and anti-boring paints and coatings on marine vessels, buoys and structures. Much of the research on metals toxicity to aquatic organisms involved freshwater species, especially fish. One of the earliest reports (Richet, 1881) compared the toxicity of various metals to fish. Other studies on metals toxicity to fish such as those by Thomas (1915), Carpenter (1927), Jones (1938), Jones (1939) and Domogalla (1935) are reviewed by Doudoroff and Katz (1950).

Most of the early studies on the effects of metals on marine organisms have examined copper and mercury toxicity. Miller (1946) determined the effects of copper on *Bugula*. Other investigations on the effects of copper and/or mercury on marine organisms are as follows: Hunter (1950) on the amphipod *Marinogammarus*; Pyefinch and Mott (1948) barnacles and their larvae; and Waksman et al (1942) and Starr and Jones (1957) on marine bacteria. Corner and Sparrow (1956) presented evidence that copper affects the respiration of crustaceans, whereas the mode of action of mercury was different. Barnes and Stanbury (1948); Pyefinch and Mott (1948), Hunter (1950); Doudoroff and Katz (1953) and Corner and Sparrow (1956) indicated that copper and mercury were synergistically toxic to various aquatic organisms.

In 1904, Moore and Kellerman used copper sulfate to control the growth of freshwater algae, especially phytoplankton, in reservoirs. Subsequently, the use of copper sulfate to control freshwater algae has been a standard practice (Whipple, 1947; Hale, 1950; Monie, 1956; and Fisher, 1956). Greenwald (1956, 1957) lists over 200 publications on the use of copper and other metals to control aquatic plants, especially algae. Palmer and Maloney (1955) screened 76 compounds (including Cu, Zn, Ag and Hg compounds) for their toxicity to six cultures of freshwater planktonic algae. Maloney and Palmer (1956) determined the toxicity of six of the more toxic of these compounds (including Cu and Zn compounds) to cultures of 30 freshwater species of Chlorophyta, Cyanophyta and Bacillariophyceae. In tests employing nine metals (Cd, Cu, Co, Pb, Hg, Ag, Se, Ba and Ni) and four freshwater phytoplankton organisms--*Chlorella, Scenedesmus, Haematococcus,* and *Chlamydomonas,* Hutchinson, 1973; and Stokes, *et al,* 1973, determined that (1) silver inhibited the growth of batch cultures of the most sensitive organism--*Chlorella,* at levels of 0.005 ppm; (2) all metals except barium and lead were toxic at concentration of 0.5 ppm or less; and (3) some combinations of metals, e.g. Cu and Ni were synergistically toxic and other combinations, e.g. Cd and Se were less toxic. The relative toxicity of the metals to *Chlorella* were as follows:

Ag>Cd>Cu>Se>Ni>Co>Ba>Pb

and to *Scenedesmus* were as follows:

Ag>Cd>Ni>Se>Cu>Ba>Pb

Whitton's (1970) review of heavy metal toxicity to freshwater algae emphasized the effects of environmental factors on metals toxicity and discussed such factors as chelation, salinity, sediments and adaption as possible causes of observed variabilities of metal toxicity. Recent studies of metals toxicity evaluated long-term effects on growth and other physiological parameters (Harriss *et al,* 1970; Matson *et al,* 1972; Tomkins and Blinn, 1976; Blinn *et al,* 1977.

Although metallic compounds have been used to control freshwater phytoplankton and many studies have been conducted on their relative toxicity to the forms and factors that cause variabilities of toxicity, the toxicity of metals to marine plants has not been as thoroughly investigated. Miyaki (1935) used copper sulfate in an attempt to control a dinoflagellate bloom in a Japanese estuary. Shilo *et al,* 1955, determined the values of pyridylmecuric acetate and copper sulfate in the control of *Prymnesium paruum* in brackish water ponds in Israel. Copper, silver, mercury and gold were the most toxic elements tested to *Gymnodinium breve* cultures (Wilson 1958). Rounsetell and Evans (1958) stated that "20 pounds of $CuSO_4$ crystals per acre will destroy the red tide organism, but will give only temporary relief for a period of 10-14 days...". Marvin and Proctor (1964) listed some metallic and organo-metallic compounds to be among the most toxic of 4,000 plus compounds screened for their toxicity to *Gymnodinium breve.* Ukeles (1962) determined that urea and ethyl mercury compounds were the most effective of 17 compounds tested in the inhibition of growth of axenic cultures of *Monochrysis lutheri, Dunaliella euchlora, Chlorella* sp., *Protococcus* sp. and *Phaeodactylum tricornutum.*

Many of the recent studies on metals toxicity to marine phytoplankton have emphasized physiological effects, such as growth and carbon-14 uptake, rather than acute toxicity. Mandelli (1969) determined that between 0.03 and 0.26 mg. cu. per liter inhibited the growth of nine marine phytoplankton species and that the uptake of copper by these cultures occurred mostly within the first 30 minutes after exposure. Phytoplankton were inhibited by copper concentration progressively more as the exposure time was increased from 20 to 60 minutes (Steeman-Nielsen *et al,* 1970) and growth depression of a diatom by copper varied from one natural seawater sample to another (Erickson, 1970). This variable attribute of different seawater samples was employed by Davey *et al* (1973) to estimate the copper complexation capacity of seawater. Mercury (as phenylmercuric acetate) concentrations as low as 0.06 mg. per liter was inhibitory to three phytoplankton species (Nuzzi, 1972).

These results emphasize the possible deleterious effects of very low metal concentrations and some of the factors that effect metal toxicity.

On the other hand, certain metals are known to be essential for the growth of marine phytoplankton and their availability often determines the level of primary productivity. Harvey (1955) listed Fe, Mn, Cu, Zn, Mo and Co as probable essential elements for phytoplankton growth. Hutner *et al* (1950) discussed the roles of metals in protisan metabolism. The role of metals in phytoplankton growth and the availability of metals in seawater, as nutrients or as toxicants is emphasized by the studies of Thomas, 1959; Menzel and Ryther, 1961; Johnston, 1963, 1964; Tranter and Newell, 1963; Wilson, 1966 and Barber, 1973. Davies (1970) considered that all phytoplankton require organo-iron complexes to synthesize chlorophyll. The potential toxicity of very low concentrations of metals and the essentiality and/or uptake of these metals indicates that one or more systems make metals available and either that system or another system reduces the ionic concentration to which the organisms are exposed in the medium.

Physical and chemical factors alter the toxicity of metals according to several investigators. Moore and Kellerman (1905) proposed that the amount of copper required to control freshwater algae should be increased 2.5% for each degree below 15C and decreased the same amount for each degree above 15C, and the amount of copper employed for control should be increased in water with high alkalinity and/or high organic content. Whipple (1947) states that the toxicity of copper in freshwater depends on the temperature, organic matter and suspended material of the water. Shilo (Shelubsky, 1954) emphasized that temperature changes do not alter copper toxicity, but that organic matter resulting from phytoplankton growth reduces toxicity. Oxygen content, pH and alkalinity affect the solubility of metals in seawater (Wood Hole Oceanographic Institution, 1952) and thus affect metals toxicity. Barnes and Stanbury (1948) discussed the formation of copper complexes by chlorides, sulfates and carbonates. Since dissolved O_2, pH, alkalinity, chlorides, sulfates and carbonates of seawater are related to salinity, the toxicity of metals may be salinity-dependent. On the other hand, the composition of oceanic and estuarine water may be markedly different in that estuarine waters may exhibit variable major ionic ratios as well as variable total dissolved solids. Although the effects of differences in ionic ratios on estuarine organisms is considered to be of less significance than the total salt content (Kinne, 1964) little information is available on the combined effects of heavy metals and variable ionic ratios Provasoli *et al* (1954).

The program included six major investigation areas titled in the sections as follows:

2. The acute toxicity of nine metals (Ag, Cr, Cu, Fe, Hg, Mo, Ni, V and Zn) to cultures of four marine phytoplankton organisms (*Glenodinium halli* Frudenthal and Lee, *Gymnodinium splendens* Lebour, *Isochrysis galbana* Parke and *Thalassiosira pseudonana* (3H) Hasle and Heimdal; = (*Cyclotella pseudonana* Husfedt).

3. The residual toxicity of nine metals to *G. splendens, I. galbana, T. pseudonana* and *G. halli*.

4. The effects of temperature and salinity on the acute toxicity of metals to *G. splendens, I. galbana* and *T. pseudonana*.

5. Some instantaneous effects of Hg, Cu and Cr on the photosynthetic rate of *G. splendens, T. pseudonana* and *I. galbana*.

6. The effects of light intensity, temperature, cell density and chronic exposure to mercury and chromium on two marine phytoplankters in continuous culture (Turbidostat tests).

7. The chronic toxicity of Hg^{++} $Cr^{+++}$and $Ni^{++}$to *G. halli* and *I. galbana* (Chemostat tests).

SECTION 2

ACUTE TOXICITY OF NINE METALS TO MARINE PHYTOPLANKTON CULTURES

MATERIALS AND METHODS

Bacteria-free cultures of *G. splendens* Lebour, *G. halli* Frudenthal and Lee, *I. galbana* Parke and *T. pseudonana* Hasle and Heimdal were grown in a chelate-free artificial seawater medium (TABLE 1) after it had passed through a Chelex 100 column (Davey *et al*, 1970). Glassware used in toxicity tests was silicone-coated. *T. pseudonana* grew better if the medium received additional silica (TABLE 1). Although it was not employed for medium of these tests, *G. splendens* and *G. halli* grew better if the medium contained Tris (hydroxy) methyl aminomethane (0.4 grams/L; pH 8.1; Matheson Coleman and Bell).

A standard temperature and salinity of 20C and 28 ppt were employed in all tests with *I. galbana* and *T. pseudonana* and a temperature of 28C and 28 ppt was employed as control levels for *G. splendens, I. galbana* (Freeberg, 1976), *T. pseudonana* (3H, Guillard and Ryther, 1962 and *G. halli* (Wilson, unpublished data). Screw-capped 125-ml microfernbach and 16 x 125mm Corning disposable test tubes with polypropylene thimble closures were employed in all tests. Preliminary results indicated that metals were more toxic in culture portions of 50-ml or more than in 10-ml portions. These effects were apparently a surface-volume relationship and the use of new test tubes and other glassware used in toxicity tests were rinsed and filled with triple glass-distilled water and autoclaved at 20 psi for 20 minutes before and after being silicone-coated.

Triplicate 10-ml culture portions were employed for each concentration of the test metal. Dilutions of metal solutions, prepared with distilled water, were added in 0.1-ml amounts to the test tubes prior to adding the culture portions. Cultures were enumerated on a Model B Coulter Counter and examined microscopically prior to use in tests.

Test portions were examined after exposure periods of 24 and 48 hours and relative chlorophyll *a* measurements were made, *in vitro*, with a Turner Model 111 Fluorometer (Lorenzen, 1966) at these times. The door on the Fluorometer was modified to accommodate the 125mm-long test tubes. All test portions were arranged in a single row which was a uniform distance from the uniform bright portion of 40-watt, cool-white, fluorescent light. Light intensity measurements were monitored with a Gossen Luna-Pro Light Meter.

TABLE 1. COMPOSITION OF CHELATE-FREE ARTIFICIAL SEAWATER MEDIUM WHICH SUPPORTED THE BEST GROWTH OF *I. galbana*, *T. pseudonana*, *C. nana*, *G. splendens* and *G. halli*

Component	Amount per liter
NaCl	24.0 g
KCl	0.6 g
$MgCl_2 \cdot 6H_2O$	4.5 g
$MgSO_4 \cdot 7H_2O$	6.0 g
$CaCl_2 \cdot 2H_2O$	0.7 g
K_2HPO_4	10.0 mg
KNO_3	10.0 mg
Metals T	1.0 ml (1)
Vitamin B_{12}	1.0 µg
Thiamine HCl	10.0 mg
Biotin	0.5 µg
Sulfides	5.0 ml (2)
Adenine Sulfate	1.0 mg
$NaHCO_3$	0.2 g (3)

(1) Metals T added after passing medium through Chelex 100 column

Metal T	mg per milliliter
Fe (as $FeCl_3 \cdot 6H_2O$)	0.05
B (as H_3BO_3)	0.05
Se (as H_2SeO_3)	0.01
V (as NH_4VO_3)	0.005
Cr (as K_2CrO_4)	0.002
Mn (as $MnCl_2 \cdot 6H_2O$)	0.01
Mo (as $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$)	0.01
Cu (as $CuCl_2 \cdot 2H_2O$)	10.001
Zn (as $ZnCl_2$)	0.005
Ti (as TiO_2)	0.05
Si (as $Na_2SiO_3 \cdot 9H_2O$)	0.05
Zr (as $ZrOCl_2 \cdot 8H_2O$)	0.02
Ba (as $BaCl_2$)	0.01

(2) Sulfides: NH_4Cl 0.2 g; KH_2PO_4 0.1 g; $MgCl_2 \cdot 6H_2O$ 0.04 g; $NaHCO_3$ 0.2 g; $Na_2S \cdot 9H_2O$ 0.15 g. Raise to one liter with distilled water.

(3) $NaHCO_3$ was added prior to autoclaving. Occasionally this medium turned cloudy (precipitate) on autoclaving (15 lbs., 15 min.) but cleared after 3 or 4 days equilibration with atmospheric CO_2. The pH also increased to 8.4-8.8 during autoclaving but returned to 8.0-8.2 as the medium cleared. Aseptic filtration was employed after the medium cleared.

(continued)

TABLE 1 (continued)

Two modifications of artificial seawater medium employed in this study.

Constituent	Gates & Wilson (per liter)	NH-15 (per liter)	NH-15 CF (per liter)
EDTA*	10.0 mg	10.0 mg	-
TRIS[†]	100.0 mg	400.0 mg	-
Fe (as $FeCl_3 \cdot 6H_2O$)	250.0 μg	289.4 μg	57.9 μg
B (as H_3BO_3)	250.0 μg	262.2 μg	52.4 μg
Se (as H_2SeO_3)	50.0 μg	30.6 μg	6.1 μg
V (as NH_4VO_3)	25.0 μg	26.1 μg	5.2 μg
Cr (as K_2CrO_4)	10.0 μg	14.8 μg	3.0 μg
Mn (as $MnCl_2 \cdot 6H_2O$)	50.0 μg	51.4 μg	10.3 μg
Ti (as TiO_2)	250.0 μg	249.4 μg	49.9 μg
Si (as $Na_2SiO_3 \cdot 9H_2O$)	250.0 μg	247.2 μg	49.4 μg
Zr (as $ZrOCl_3 \cdot 8H_2O$)	100.0 μg	56.6 μg	11.3 μg
Ba (as $BaCl_2$)	50.0 μg	49.5 μg	9.9 μg
Sulfides Mix	1.0 ml	5.0 ml	5.0 ml
$NaHCO_3$	-	-	200.0 mg

*2-Amino-2-hydroxymethyl-1, 3-propanediol

†Disodium ethylene diaminotetraacetate

The modification designated NH-15 was employed for the assessment of physical factors on growth. Chelate-free medium (NH-15 CF) was used to determine the chronic effects of Hg, Ni and Cr. The media were prepared (8 L amounts) in 12-liter Pyrex bottles, filtered through Whatman's GF/A glass fiber filters, and autoclaved at 15 psi for 1 hour. A sterile $NaHCO_3$ solution was added to NH-15 CF after autoclaving when the temperature of the medium had decreased below 35C. This solution was prepared by placing a 250 ml screw cap Erlenmeyer flask containing 13.5 grams of $NaHCO_3$ in an oven at ± 30 minutes, followed by an aseptic addition of 150 ml of sterile water to the flask.

Rigid control of light quality and intensity was required to avoid light elicited variations of the chlorophyll *a* measurements. This system is an excellent method of evaluating growth without disturbing the culture population and measurements can easily be made often, if necessary. Examination of the tubed test-portions were made also with an American Optical inclined stage stereoscopic microscope with 3x and 10x magnification.

Experiments for the determination of the toxicity of various materials to phytoplankton cultures had a major advantage over toxicity experiments involving most organisms in that a large number of experimental containers each with approximately 10^3 to 10^7 organisms/L can be utilized for each test condition. On the other hand, it was frequently difficult to detect toxic effects on phytoplankton culture populations. This was especially true with organisms that are non-motile and do not lyse readily after exposure to what proved to be a lethal concentration of a material. This is the case with the diatom, *T. pseudonana* and this is one reason we adopted the fluorometric method to detect *in vitro* change in chlorophyll *a*. In addition, we extended the total period of observation from 24 hours to 48 hours. Although these methods were employed and care was exercised to prevent variable results, variations in the toxicity of a metal did occur between experiments (Figure 1). This variability involved experiments with *G. splendens* in which motility was used to evaluate toxicity effects. Primarily because of such variations, the minimum toxic concentration, the maximum non-toxic concentrations and the minimum concentration that appeared to cause the lysis of most cells in a test portion are included as levels of toxicity in this report. The term "toxic concentration" as used in this report on acute toxicity is in the following account.

In theory, the addition of a specific amount of substance to a phytoplankton culture portion may result in one of eight conditions, or more, after a relatively short period of exposure (e.g., 48 hours) as follows:

1. Effective culture growth exceeds that of a control portion (one that received no added test material).
2. Effective culture growth was similar to that of the control portion.
3. Growth greatly exceeds mortality but effective growth is less than the control.
4. Growth slightly exceeds mortality or effective growth is much less than the control.
5. Growth is similar to mortality, or there is no effective growth.
6. Growth is less than mortality or there is slight effective mortality.
7. Growth is much less than mortality or there is high effective mortality.
8. Total or almost total mortality of the test population.

It is possible for all of these events to occur in different test portions of a single bioassay (Figure 2).

In experiments conducted during this program in which the population levels of test portions of a culture were followed for 48 hours or longer

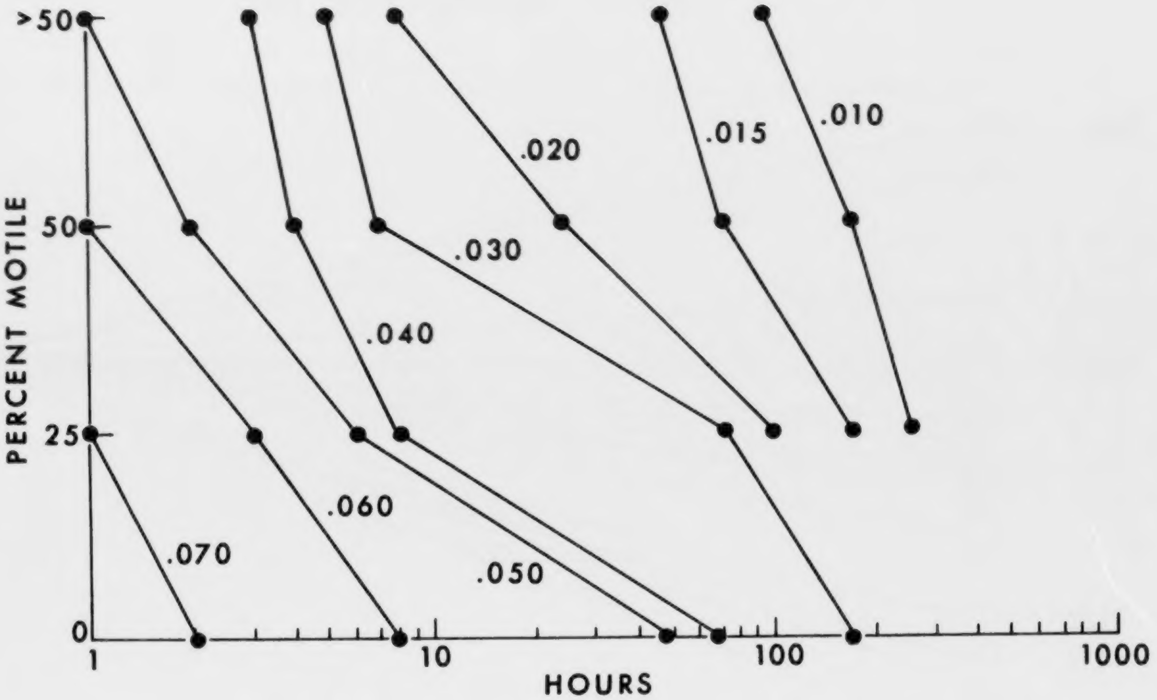

Figure 1. Variability of Cu toxicity to *G. splendens* (values in mg/L).

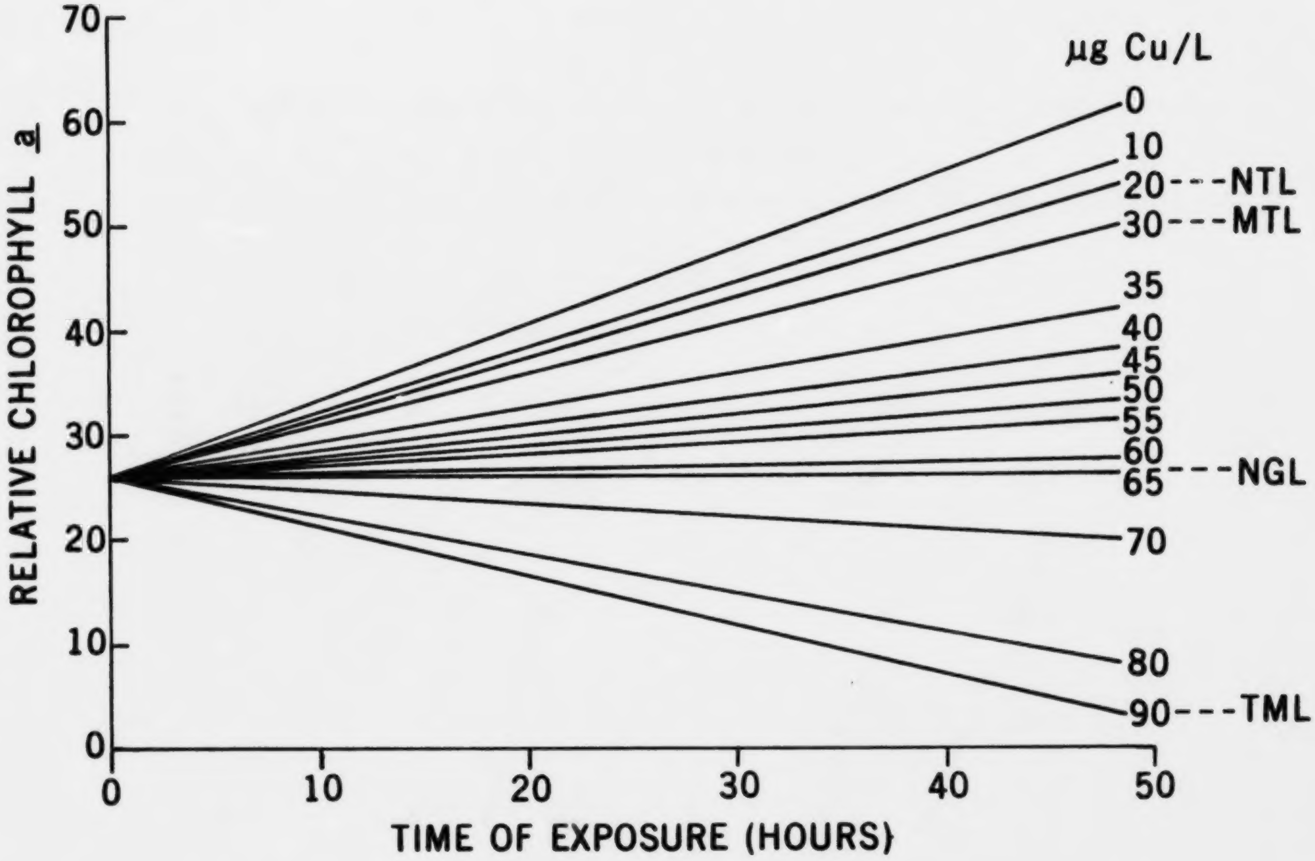

Figure 2. The relationship of MTL, NTL, NGL and TML to the toxicity of Cu to *G. splendens* (results from 11 experiments).

on a daily schedule by determining the relative chlorophyll *a, in vitro,* the following pattern of results occurred if the test culture was growing at the time the experiment was initiated.

1. Populations in control portions continued to grow, i.e., the chlorophyll *a* content was higher after 48 hours than at the time the test was started.

2. Populations in certain test portions with low metal addition continued to grow. In some cases, the culture portion grew faster or the same as the control portion.

3. Populations in test portions that received increasingly higher metal additions than the control had progressively lower final populations.

4. The population levels of some test portions did not change greatly during the test period.

5. Populations that received progressively higher metal additions than those mentioned in Items 1 through 4 decreased significantly and in some cases they decreased to the level of "0" relative chlorophyll *a* value with the most sensitive Fluorometer window setting (30X).

Although the population level of a culture may have been high, if the culture was not growing at the time an experiment was initiated, the population level of all test portions and the controls usually decreased. Since the population of the culture portion that received no addition of the test material (control portion) may decline more than those of test portions that received growth inhibiting levels of a substance, and this often occurs, the assay results are not a valid measure of toxicity. Indeed, such assays conducted with natural populations could be misleading.

As a result of these developments, only actively growing cultures were used in the acute toxicity experiments. Cultures were established by transferring large inocula into fresh medium 3 to 5 days (*T. pseudonana* and *I. galbana*), 4 to 6 days (*G. halli*) or 14-18 days (*G. splendens*) before a culture was to be used in an experiment. Based on this procedure and the previously described observations, the toxicity terms used in this report are as follows:

Minimum Toxic Level (MTL) was that level of minimum metal addition in which the increase of the chlorophyll *a* content of the test population was approximately 65 percent of the increase of the control population during the 48-hour test period.

Maximum Non-Toxic Level (NTL) was that level of maximum metal addition at which the increase of relative chlorophyll *a* of the test population was significantly greater than 65 percent of the increase in the control population during the 48-hour test period. Since results of more than one experiment are included in these determinations this value can be either higher, lower or the same as the MTL.

No Growth Level (NGL) that level of metal addition in which the relative chlorophyll *a* of the test population changes less than 5 percent during the 48-hour test period.

Minimum Total Mortality Level (TML) that level of metal addition at which the chlorophyll *a* of the test population decreased during the 48-hour test period to a fluorometric reading of less than 5 (on scale of 0 to 100) with the most sensitive (30X) window of the Fluorometer.

These definitions are not entirely correct. Most results indicate that there are progressively lesser degrees of metal inhibition associated with progressively lower metal additions (Figure 2). The end-point of inhibition is difficult to establish. Often test portions that receive small specific amounts of two metals in one experiment may grow to a lower terminal population level than the control portion, but in another comparable experiment the terminal population level may be higher than that of the control. Also, the four toxicity levels (Figure 2) will obviously be progressively higher (NTL>MTL>NGL>TML), if only results of a single experiment are considered, but combining results of three or more experiments often resulted in a lower defined level having the same toxic effects as the next even higher toxicity level. Although these levels were arbitrarily selected, they represent toxicity levels that are basic to evaluating the effects of a substance on a phytoplankton culture.

RESULTS

The four organisms were not equally inhibited by any one of the nine metals (TABLE 2). The toxicity levels of some metals were similar for two organisms (e.g., the LMTL of the nine metals to *G. splendens* and *G. halli*), but not as many as three organisms. The lower MTL of Ni were nearer the same for all four organisms (0.1 to 0.5 mg/L) than were those of any other metals (TABLE 2). On the other hand, the toxicity levels of some metals differed by an order of magnitude or more between organisms. Also, based on the LMTL and LNGL, with the exception of Ni and Mo, the metals were much more toxic to the two dinoflagellates than to *I. galbana* and *T. pseudonana* cultures.

The nine metals were more toxic to *G. splendens* and *G. halli* than to *T. pseudonana* and least toxic to *I. galbana*. Although 13 of the 18 LMTL and LNGL of the nine metals were higher for *I. galbana* than for *T. pseudonana*, all nine of the LTML for *I. galbana* were higher than those for *T. pseudonana*. The toxicity levels of the nine metals to the two dinoflagellates were similar. If there was a difference in their sensitivity to these metals, *G. splendens* was possibly more susceptable to lower additions of more of the metals than was *G. halli*.

Despite these observed differences in the toxicity levels between organisms, there were broad patterns of toxicity levels of groups of the metals to all four organisms. Based on the LMTL and LNGL of the nine metals to the four organisms, three groups of metals can be arranged (TABLE 3). Although there are exceptions and there are overlapping values, the means of

TABLE 2. ACUTE TOXICITY OF NINE METALS TO CULTURES OF FOUR MARINE PHYTOPLANKTON ORGANISMS (VALUES IN MG/L)

G. splendens
Toxicity Level

Metal	MTL		NTL		NGL		TML		Number of Experiments
	L*	H	L	H	L	H	L	H	
Hg	.005	.09	.002	.08	.02	.1	.04	.3	9
Cu	.01	.1	.005	.09	.01	.1	.07	.2	18
Ag	.005	.04	.002	.03	.01	.08	.02	.1	14
Zn	.05	1.0	.02	.8	1	5	2	10	9
Cr	.05	.8	.02	.5	.1	7	.5	25	9
Fe	.2	5	.1	4	.5	25	1	30	8
Ni	.2	6	.1	5	1	15	15	20	10
V	1	20	.5	18	2	25	10	25	10
Mo	2	35	1	30	5	70	10	200	9

I. galbana
Toxicity Level

Metal	MTL		NTL		NGL		TML		Number of Experiments
	L	H	L	H	L	H	L	H	
Hg	0.1	.9	.08	.8	.2	1	.3	5	19
Cu	.05	.2	.02	.3	.1	1	1	>2	28
Ag	.02	.1	.01	.2	.05	1	.7	>5	28
Zn	.5	4	.2	3	1	7	7	>10	14
Cr	.5	10	.4	5	.6	>40	>40	>40	12
Fe	1	25	.5	20	6	30	>35	>35	17
Ni	.5	3	.2	2	.5	>50	>50	>50	12
V	20	30	18	40	50	>50	50	>50	12
Mo	10	>300	20	>300	20	>300	>300	>300	7

*Two levels of toxicity (low-L) and (high-H) are designated for each toxicity level. The low level (L) is the minimum addition of the metal in the total number of experiments (right column) that had the designated toxicity to occur with the metal addition shown and the high level (H) is the maximum metal addition that produced this toxicity effect.

(continued)

TABLE 2 (continued)

T. pseudonana
Toxicity Level

Metal	MTL		NTL		NGL		TML		Number of Experiments
	L	H	L	H	L	H	L	H	
Hg	.06	.5	.05	.1	.08	1	.08	1	10
Cu	.06	.08	.05	.07	.08	.5	.1	.5	10
Ag	.04	.08	.03	.06	.08	.2	.08	.5	14
Zn	.1	.5	.08	.4	.5	2	1	20	10
Cr	.02	.08	.01	.05	.08	.6	.1	>10	9
Fe	8	>20	7	>20	20	>20	>20	>20	10
Ni	.1	.4	.05	.2	.2	10	.2	>100	10
V	10	30	5	30	10	40	20	40	10
Mo	1	100	.5	80	10	>100	60	>100	10

G. halli
Toxicity Level

Metal	MTL		NTL		NGL		TML		Number of Experiments
	L	H	L	H	L	H	L	H	
Hg	.02	.05	.01	.02	.03	.1	.05	>.1	8
Cu	.005	.14	.002	.12	.01	.16	.05	>5	10
Ag	.005	.04	.002	.02	.01	.07	.05	2	6
Zn	.02	.2	.01	.1	.2	2	5	50	5
Cr	.02	.5	.01	.2	.1	.5	.2	>20	10
Fe	.5	5	.2	2	2	>10	10	100	5
Ni	.2	5	.1	2	.5	20	2	50	6
V	2	20	1	10	10	25	50	100	4
Mo	2	25	1	20	20	50	50	>100	4

TABLE 3. THE LOW MINIMUM TOXICITY LEVEL (LMTL-1), LOW NO GROWTH LEVEL (LNGL-2) AND LOW TOTAL MORTALITY LEVEL (LTML-3) OF NINE METALS TO FOUR PHYTOPLANKTON CULTURES (VALUES IN MG/L)

	ORGANISM			
Metal	*T. pseudonana*	*I. galbana*	*G. splendens*	*G. halli*
Hg 1	0.06	0.1	0.005	0.02
2	0.08	0.2	0.02	0.03
3	0.08	0.3	0.04	0.05
Cu 1	0.06	0.05	0.01	0.005
2	0.08	0.1	0.01	0.01
3	0.1	1.0	0.07	0.05
Ag 1	0.04	0.02	0.005	0.005
2	0.08	0.05	0.01	0.01
3	0.08	0.7	0.02	0.05
Zn 1	0.1	0.5	0.05	0.02
2	0.5	1.0	1.0	0.2
3	1.0	7.0	2.0	5.0
Cr 1	0.2	0.5	0.05	0.02
2	0.08	0.6	0.1	0.1
3	0.1	>40.0	0.5	2.0
Ni 1	0.1	0.5	0.2	0.2
2	0.2	0.5	1.0	0.5
3	0.2	>50.0	15.0	2.0
Fe 1	8.0	1.0	0.2	0.5
2	20.0	6.0	0.5	2.0
3	>20.0	>35.0	1.0	10.0
V 1	10.0	20.0	1.0	2.0
2	10.0	50.0	2.0	10.0
3	20.0	50.0	10.0	50.0
Mo 1	1.0	10.0	2.0	2.0
2	10.0	20.0	5.0	20.0
3	60.0	>300.0	10.0	50.0

the LMTL of the three groups progressively differed by a six-fold factor as follows: Group I--0.03 mg/L, Group II--0.18 mg/L, Group III--1.3 mg/L and Group IV--6.0 mg/L. A questionable feature of this group arrangement involves Fe and Ni. As mentioned previously, the variation of the LMTL of Ni for the four organisms was 0.1 to 0.5 mg/L (Mean-0.2 mg/L), the smallest range of the nine metals. On the other hand, the Fe LMTL varied from 0.2 to 8.0 mg/L (Mean-1.2 mg/L); the largest toxicity range of these metals. The high tolerance of *T. pseudonana* to Fe additions was the major contributor to this variability. If either the LMTL of *G. splendens* and *G. halli* or the LMTL values for *I. galbana* and *T. pseudonana* are combined, the proposed groups are less variable. If the molecular concentration of the toxicity levels of the nine metals are compared, the same group arrangement resulted (TABLE 4). The results presented above pertained to the low levels of toxicity observed in the numerous tests conducted. Although these lower values represent levels of toxicity that must be of primary consideration, there are other results, (e.g., the high levels of toxicity), that should be considered for a more complete evaluation of the tests.

The high levels of toxicity (HMTL, HNGL and HTML) were approximately one order of magnitude higher than the low values previously presented (TABLE 5). For example, Group I metals had HMTL from 0.04 to 0.9 mg/L whereas the LMTL values were 0.005 to 0.1 mg/L and Group I mean HMTL and LMTL were 0.19 and 0.03 mg/L, respectively. The greatest differences in the low and high levels of toxicity that occurred in the tests were in the Group II metals--Zn and Cr. For example, values for the four organisms were from 0.08 to 10.0 mg/L compared to the LMTL of 0.02 to 0.5 mg/L. Although some tests were repeated as many as five times in attempts to determine the cause(s) or reduce this variability, the variability continued and the causes remained unknown. In addition, although the general trend of increased toxicity according to group number was observed with the high toxicity levels, the HMTL, HNGL and HTML of the Group II and Group III metals overlapped more than did the low toxicity level values. The higher variability and more ill-defined groups should probably be expected, as this was usually the case as the toxicity of a substance was less.

TABLE 4. GROUP LIST OF NINE METALS BASED ON THEIR GRAVIMETRIC (MG/L) AND MOLECULAR μg AT/L WEIGHTS OF COMBINED LMTL AND LNGL ACUTE TOXICITY VALUES TO *G. halli*, *G. splendens*, *I. galbana* and *T. pseudonana*

		Toxicity Level			
		LMTL		LNGL	
	Metal	MG/L	μg AT/L	MG/L	μg AT/L
Group I	Ag	0.005-0.04	0.04-0.37	0.01-0.08	0.09-0.74
	Cu	0.005-0.06	0.08-0.94	0.01-0.08	0.16-1.27
	Hg	0.005-0.1	0.03-0.50	0.02-0.08	0.10-0.40
Group II	Cr	0.02-0.5	0.39-9.6	0.08-0.6	1.5-11.5
	Zn	0.02-0.5	0.31-7.6	0.2-1.0	0.31-15.3
	Ni	0.1-0.5	1.7-8.5	0.2-1.0	3.4-16.9
Group III	Fe	0.2-0.5	1.7-8.5	0.5-20.0	8.9-357
	V	1.0-20.0	19.6-392	2.0-50.0	39.2-980
	Mo	1.0-10.0	10.4-104	5.0-20.0	52.0-208

TABLE 5. HIGH MINIMUM TOXICITY LEVEL (HMTL-1), HIGH NO GROWTH LEVEL (HNGL-2) AND HIGH TOTAL MORTALITY LEVEL (HTML-3) OF NINE METALS TO FOUR MARINE PHYTOPLANKTON CULTURES

		Tp	*Ig*	*Gs*	*Gh*	Toxicity Level
Hg	1	0.5	0.9	0.09	0.05	1
	2	1.0	1.0	0.1	0.1	2
	3	1.0	5.0	0.3	>0.1	3
Cu	1	0.08	0.2	0.1	0.14	1
	2	0.5	1.0	0.1	0.16	2
	3	0.5	>2.0	0.2	>5.0	3
Ag	1	0.08	0.1	0.04	0.04	1
	2	0.2	1.0	0.08	0.07	2
	3	0.5	>5.0	0.1	2.0	3
Zn	1	0.5	4.0	1.0	0.2	1
	2	2.0	7.0	5.0	2.0	2
	3	20.0	>10.0	10.0	50.0	3
Cr	1	0.08	10.0	0.8	5.0	1
	2	>10.0	>40.0	7.0	5.0	2
	3	>10.0	>40.0	25.0	>200.0	3
Ni	1	0.4	3.0	6.0	5.0	1
	2	10.0	>50.0	15.0	20.0	2
	3	>100.0	>50.0	20.0	50.0	3
Fe	1	>20.0	25.0	5.0	5.0	1
	2	>20.0	30.0	25.0	>10.0	2
	3	>20.0	>35.0	30.0	100.0	3
V	1	30.0	30.0	20.0	20.0	1
	2	40.0	>50.0	25.0	25.0	2
	3	40.0	>50.0	25.0	100.0	3
Mo	1	100.0	>300.0	35.0	25.0	1
	2	>100.0	>300.0	70.0	50.0	2
	3	>100.0	>300.0	200.0	>100.0	3

SECTION 3

RESIDUAL TOXICITY OF NINE METALS TO *G. splendens, I. galbana, T. pseudonana and G. halli*

Toxicological reports give accounts of the uptake of sufficient quantities of a substance in a short time period to produce subsequent mortality or metabolic inhibition. In the evaluation of the toxicity of metals to phytoplankton, a consideration which had not received attention was whether or not a phytoplankton population could be exposed to a toxic metal concentration and subsequently grow at a normal rate and if so, what was the highest concentration that would not permanently restrict subsequent growth and how did this value compare to the acute toxicity level. Mandelli (1969) showed that the uptake of Cu by four phytoplankton organisms occurred in the first hour of exposure. Button and Hostetter (1977) showed that the uptake of copper by two freshwater phytoplankton organisms was fast during the first five minutes of exposure and continued at a reduced rate for a period of two hours, but there were small, if any, amounts removed from the medium afterwards. Both reports showed that the organisms released Cu after they were placed in fresh medium and the latter publication indicated that most of the copper (46% and 59%) was released within the first two hours. Thus, organisms that survived the period of exposure to a toxic metal concentration may subsequently release the absorbed material and experience no residual effect. On the other hand, a population may survive the exposure period but sustain permanent physiological damage and eventually die-off. Residual toxicity tests were conducted in an attempt to evaluate the possibility of permanent impairment to cultures of four phytoplankton organisms.

In the residual toxicity experiments with *G. splendens, I. galbana* and *T. pseudonana,* culture portions of the organisms were exposed to various concentrations of each metal for a period of 48 hours following the same procedure as described for the acute toxicity tests. After this 48-hour exposure, either 0.1 ml (*I. galbana* and *T. pseudonana*) or 0.3 ml (*G. splendens*) from each test portion was transferred to 10 ml portions of fresh culture medium (TABLE 1) and incubated for 14 days in the standard culture condition described in the previous section. If growth occurred after the transfer, the growth rates were determined from the relative chlorophyll *a* measurement that were made every 1 to 3 days. If growth rates were below normal, subcultures were made to determine if the growth of that specific population was permanently impaired. If no growth occurred in a test portion, after the transfer to fresh culture medium, it was assumed that there were no viable cells or that their growth had been permanently inhibited. The highest level in which growth occurred was recorded as the level of residual toxicity. This was done because cultures grew from many of the test portions that received the maximum additions of the metals. Residual toxicity tests with *G. halli* were conducted in this same manner except that 0.1 ml portion of the test portions were transferred to fresh culture medium after exposure to the various metal addition for period of 1, 24, 48, 72 and 144 hours.

RESULTS

In some instances cultures exposed to high metals concentrations subsequently grew at a reduced rate during the 14-day incubation period, but their normal growth after subsequent subcultures were made indicate that there was no permanent metabolic impairment. Although it could not be determined with certainty, results indicated that if some organisms survived in a test portion during the exposure period, they resumed normal growth following one or two transfers in fresh medium. Indeed, cells transferred from test portions in which no surviving cells were detected, by microscopic examination, subsequently grew. Yet, it was frequently observed in cultures of *G. splendens, G. halli* and *I. galbana* that most of the cells in a test portion lysed during the exposure period, but a few individuals survived as indicated by their motility. Other test portions that appeared by microscopic examination to contain living cells did not grow after being transferred. Fluorometric measurements often were better indicators of the presence of live cells. A relative chlorophyll *a* value of "0" with the most sensitive (30X) window setting was usually a reliable indicator that a test portion contained no living cells, but test portions that gave a measurable fluorometric reading did not always grow after being transferred.

Although metal additions were increased to relatively high levels, the organisms remained viable in over 50% of the test portions that contained the maximum concentration tested.

Higher addition than those listed (TABLES 6 and 7) were not tested because the saturation level of the metal in seawater had been greatly exceeded and precipitation of the various components of the medium could have inhibitory effects that were not directly related to the metal concentrations. Special systems are needed for each metal if concentrations as high or higher than those employed here are to be evaluated realistically.

In most cases, the residual toxicity level of each metal was much higher than the acute toxicity level (TABLES 6 and 7). The residual toxicity levels for V with *I. galbana* and Fe with *T. pseudonana* were between 10 and over 1000 times that of the LMTL values. On the other hand, the residual toxic levels were 10 times the HNGL or less in 28 of 36 cases and in 17 of 36 cases, they were 2 times the HNGL, or less. This latter consideration may indicate that the no growth level was slightly higher than the residual toxicity level or perhaps these were two different assessments of the same basic condition.

The residual toxicity levels of Ag, Cu and Hg to *G. splendens* were less (more toxic) in all cases and those of Fe, Ni, V and Mo were less in 7 of 9 cases than those of the other three organisms. This comparison may be indicative of a higher sensitivity of *G. splendens* to these metals which, except for results with *G. halli*, was indicated by the acute toxicity tests. On the

TABLE 6. RESIDUAL TOXICITY (A) OF NINE METALS TO *I. galbana*, *T. pseudonana* AND *G. splendens* (VALUES ARE IN MG/L)

Metal	*I. galbana*			*T. pseudonana*			*G. splendens*		
	A	B	C	A	B	C	A	B	C
Ag	7(8)	0.02	1	1(2)	0.04	1	0.1(0.2)	0.005	0.08
Cu	10(9)	0.05	1	20(30)	0.06	0.5	0.1(0.2)	0.01	0.1
Hg	6(5)	0.1	1	5(6)	0.06	0.2	0.2(0.3)	0.005	0.1
Zn	10*(D)	0.5	7	100*	0.1	2	20*	0.05	5
Cr	40*	0.5	>40	10*	0.2	>10	60*	0.05	7
Fe	35*	1.0	30	20*	8.0	>20	30(35)	0.2	25
Ni	50*	0.5	>50	100*	0.1	10	80(100)	0.2	15
V	100*	20	>50	100*	10	40	30(35)	1.0	25
Mo	300*	10	>300	100*	1	>100	100(200)	2.0	70

(A) The highest concentration (mg/L) of the metal to which the organisms were exposed for a period of 48 hours (i.e., the equivalent amount added to the test portion) and subsequently grew after being transferred to fresh medium. Numbers in parentheses are the lowest levels tested to which the organisms were exposed for 48 hours and did not grow after being transferred.

(B) The LMTL values of the metals from Table 2.

(C) The HNGL values of the metals from Table 2.

(D) Asterisk (*) indicates that this addition was the highest concentration tested.

TABLE 7. RESIDUAL TOXICITY (A) OF NINE METALS TO *G. halli* AFTER EXPOSURES OF 1, 24, 48, 72 AND 144 HOURS.

Metal	Hours of Exposure				
	1	24	48	72	144
Ag	10*(B)	10*	10*	5(10)	5(10)
Cu	0.2(0.5)	0.2(0.5)	0.2(0.5	0.5(1)	0.5(1)
Hg	10*	10*	10*	0.2(0.5)	0.5(1.0)
Zn	50*	50*	50*	50*	50*
Cr	200*	100(200)	100(200)	50(100)	50(100)
Fe	100*	100*	100*	100*	100*
Ni	100(200)	100(200)	100(200)	100(200)	10(20)
V	500*	500*	500*	500*	500*
Mo	500*	500*	500*	500*	500*

(A) The highest concentration (mg/L) of the metal to which *G. halli* was exposed for periods of 1, 24, 48, 72 or 144 hours (i.e., the equivalent amount added to the test portion) and subsequently grew after being transferred to fresh medium. Numbers in parentheses are the lowest levels tested to which *G. halli* was exposed for the indicated time and did not grow after being transferred.

(B) Asterisk (*) indicates that this addition was the highest amount tested.

other hand, the *G. halli* 48-hour residual toxicity results were similar to those of *I. galbana* and *T. pseudonana*.

The results of the *G. halli* residual toxicity tests with exposure periods of 1 to 144 hours indicated that a 48-hour exposure period does not always give representative toxicity results and that the uptake and release of the different metals may be different. The residual toxicity of some metals did not increase as the period of exposure increased. In fact in the case of Cu, the 1, 24 and 48 hour residual toxicity levels (0.2 mg/L) were less than the 72 and 144 hour exposures (0.5 mg/L). Considering the fast uptake of Cu (Mandelli 1969 and Button and Hostetter 1977), an exposure of one hour would be expected to be as effective as a longer exposure. A possible explanation is that the ionic concentration of Cu in the medium decreased during the 72 hour period and the cells released Cu.

The uptake of the other metals of the nine tested for which a residual toxicity level was determined (Ag, Hg, Cr, Ni) did not appear to be as fast as that of copper. There was no effect of exposures of 1, 24 and 48 hours to the maximum Ag and Hg additions (10 mg/L). A 1-hour exposure to 200 mg Ni resulted in no growth after transfer but longer exposures did not increase the toxicity, except that after 144 hours the toxicity level was 20 mg/L. Also, there were increases in the toxicity of Cr after exposures of 24 and 72 hours. These differences in the time of exposures required to elicit a toxicity level involve many features of the system such as the various possible reactions of the metal with the organism and the combined reactions of the metal, the medium and the organism.

SECTION 4

THE EFFECTS OF TEMPERATURE AND SALINITY ON THE ACUTE TOXICITY OF METALS TO *G. splendens, I. galbana* AND *T. pseudonana*

If the limits of the salinity or temperature ranges in which these three organisms grew are disregarded, the broad features of their growth response to temperature and salinity are predictable. The temperature response is such that as the temperature increases from some level below which there is no growth, growth rates increase gradually to an optimum level which extended upward approximately 6 to 12C. Further thermal increases of 4 to 6C caused a sharp decline of the growth rate. The salinity response was somewhat similar, but reversed. Growth rates increased gradually as the salinity decreased from some level above which no growth occurred. The sharp decline in growth rates in response to salinity occurred near the low end of the growth range and the optimum salinity level for growth was relatively broad. If these two general patterns are considered together, the levels of temperature and salinity near which the sharpest declines in growth occurred, high temperature and low salinity, would be the primary suspected combination of conditions that may interact detrimentally to the growth of these organisms. Secondarily, the combined effects of low temperature and low salinity may have reduced the growth rates. No attempt was made to determine the upper salinity levels that the three organisms tolerated, therefore the effects of high salinity, i.e. near the upper tolerance limits, in combination with different temperatures were not determined.

MATERIALS AND METHODS

Five walk-in constant temperature culture rooms were employed in this work. Usually the temperature in these rooms was maintained at 12C, 16C, 20C, 24C and 28C ± 1. Temperatures below 12C were established by lowering the room usually maintained at 12 and temperatures above 28 were established by altering the concentrations of the major salts (NaCl, KCl, $MgCl_2 \cdot 6H_2O$, $MgSO_4 \cdot 7H_2O$ and $CaCl_2 \cdot H_2O$) without altering their relative ratios and without changing the concentrations of the other medium components (TABLE 1).

Cultures for each temperature, salinity or temperature-salinity combinations were established by step-wise subculturing into progressively lower or higher temperatures, salinity levels and combination temperature and salinity levels. The growth rate of each of the three organisms was determined for each condition and only those combinations of temperature and salinity in which the organisms grew were employed in toxicity tests.

All other procedures and conditions employed in these experiments were the same as described previously for acute toxicity experiments except that the growth of the test portions were determined by measuring the relative chlorophyll *a, in vitro,* before the portions were delivered to the culture tubes and after 1, 24 and 48 hours exposure to the test concentrations of the metals. Counts were not made of the test portions during these experiments.

The evaluations of the effects of the metals were made by calculating the MTL values for each combination of metal concentrations and temperature, salinity or salinity-temperature combination. Also, the 50% mortality (M-50) values were determined for each of these conditions or combinations of conditions. The 50% mortality (M-50) values were determined by plotting the 48-hour fluorometer readings (arithmetic scale) for the various concentrations of the test metal (log scale) for temperature-salinity combinations on semi-log paper. The 50% mortality values was that concentration on the long-scale that corresponded to a population level on the curve that was 50% of the initial fluorometer reading (T=0) for the population. The MTL value was that concentration on the same curve that represented the 65% growth level of the population based on the initial (T=0) and final (T=48 hours) readings of the controls.

RESULTS

The temperature and salinity requirements of *G. splendens* (Sweeney, 1951; Thomas, *et al*, 1973; unpublished data); *Isochrysis galbana* (Kain and Fogg, 1958; Freeberg, 1976, unpublished) and *T. pseudonana* (Guillard and Ryther, 1972; Ryther and Guillard, 1962; Guillard and Mykelstad, 1970) have been treated in previous reports. However, the cultures we employed may have had or have developed slightly different requirements from the isolates used by the above investigators. For this reason the general features of the effects of temperature and salinity were examined and results are included in this report.

G. splendens grew within a temperature range of 16C to 33C and a salinity range of 14 ppt to 37 ppt. It did not grow at a temperature of 14C; and it grew only slightly at 33C in combination with 28 ppt salinity but not 14 ppt salinity (TABLE 8) No growth occurred in media with salinity levels of 12 ppt or less, but *G. splendens* grew well in media with 37 ppt salinity--the highest tested. Based on our results, *G. splendens* is a eurythermal, euryhaline organism that grew best at temperatures between 20C and 30C and salinity levels between 14 and 37 ppt. The upper limit of the temperature range was approximately 3C higher than that reported for a California isolate of *G. splendens* (Thomas *et al*, 1971).

I. galbana grew within a temperature range of 12C and 28C (the lowest and highest tested) and within the salinity range of 6 and 37 ppt, the lowest and highest tested. It's growth rate was low at 28C with salinity levels between 14 ppt and 37 ppt, but if the salinity was less than 12 ppt, it did not grow. *I. galbana* did not survive at 30C at any salinity level. The highest division rates occurred at temperatures from 16 to 24C and with salinity levels between 14 ppt and 24 ppt. This organism showed both eurythermal and euryhaline characteristics and it was tolerant of short-term temperature and salinity changes. Of the conditions tested, temperature levels of 28C and higher were the most inhibitory.

T. pseudonana (clone 3H) adjusted to progressively lower salinity levels down to the lowest tested (3 ppt) and grew well at all temperatures and salinity combinations employed. This organism grew best within broader ranges of temperature and salinity than did *G. splendens* and *I. galbana*. It's maximum growth rates were with salinity levels between 10 ppt and 28 ppt and with temperatures between 12C and 28C. The minimum growth occurred with high temperatures (24-28C) and low salinity (3 ppt-5 ppt) combinations. This isolate had euryhaline and eurythermal attributes.

TABLE 8. EFFECTS OF TEMPERATURE AND SALINITY ON THE GROWTH RATES (K) OF *G. splendens, I. galbana* and *T. pseudonana*

				G. splendens				
T C	12	16	20	24	28	30	32	33
S ppt								
10	CD*	NG†	NG	CD				
12	CD	NG	NG	CD				
14	CD	.33‡	.41	.43	.31	.29	.2	CD
16	CD	.38	.44	.48	.42	.35	.18	
18	CD	.35	.41	.40	.37	.43	.20	
20	CD	.27	.49	.5	.48	.42	.23	
24	CD	.25	.42	.45	.51	.53	.20	
28	CD	.29	.28	.29	.38	.48	.25	.16
37	CD	.2	.43	.47	.31	.51		

* CD = controls died.

† NG = controls lived, but there was no growth.

‡ K values = numerical values representing growth/cell/day.

(continued)

TABLE 8 (continued)

			I. galbana			
T C	12	16	20	24	28	30
S ppt						
6	.53	.46	0.57	.7	CD	
9	.50	.58	.74	1.0	CD	CD
12	.61	.57	.87	.99	NG	CD
14	.68	1.0	1.0	1.1	0.36	CD
16	.61	.75	.94	1.0	0.34	CD
20	.75	.84	1.1	1.1	0.36	CD
24	.73	0.83	.92	0.94	0.36	CD
28	.71	0.87	1.0	1.1	0.23	CD
37	.68	.79	.8	1.1	0.17	CD

			T. pseudonana		
3	0.71	0.72	0.69	0.58	0.45
5	.62	.68	0.72	.67	0.49
7	.69	.70	.66	.72	.42
10	.60	.68	.79	.77	.88
14	.81	.75	.78	.94	.99
21	1.0	.92	.91	1.2	1.4
28	.65	.75	.98	.77	.95

Temperature Effects on Acute Metals Toxicity to *G. splendens*.

Results of toxicity tests of the nine metals to *G. splendens* grown in medium with 28 ppt salinity and temperatures of 16C, 20C, 24C and 28C did not show that there was a general effect of these temperatures on metals toxicity. The toxicity of Ni and Zn increased as the temperature was increased (TABLE 9) but temperatures of 16C to 28C had no effect on the toxicity of V, Cu, Mo, Hg and Ag.

There were no effects of temperature on the toxicity of Fe and Cr to *G. splendens*. Concentrations between 0.1 and 1.0 mg/L of these metals apparently stimulated the growth of this organism. It was not possible to assign the possible effect of this stimulatory activity to the final experimental results. *G. splendens* Cr-LMTL was at 28C but the Cr-HMTL was at 24C. Perhaps this organism was less susceptible to metals at temperatures that were optimum for growth. Ni, V, Fe, Cr, Cu and Hg were less toxic at 24C than at 28C, but the M-50 value for these metals did not show similar results. On the other hand, the MTL values for 7 of the 9 metals were higher (less toxic) at 24C than at 16C. The two exceptions were Ni and Zn.

TABLE 9. THE EFFECTS OF TEMPERATURE ON THE TOXICITY OF METALS TO *G. splendens* (VALUES IN MG/L)

Metal	Ag		Cu		Hg	
Temperature	M-50	MTL	M-50	MTL	M-50	MTL
16C	0.032	0.0075	0.095	0.01	0.029	0.012
20C	0.054	0.018	0.086	0.017	0.038	0.026
24C	0.035	0.013	0.094	0.047	>0.05	0.023
28C	0.041	0.013	0.045	0.032	>0.05	0.019
	Ni		Cr		Zn	
16C	>20.0	1.0	>1.0	0.012	8.0	0.22
20C	>20.0	0.95	>1.0	0.012	4.5	0.24
24C	5.9	0.56	>1.0	0.016	2.8	0.11
28C	3.5	0.13	>1.0	<0.01	1.5	0.12
	Fe		V		Mo	
16C	17.5	2.0	>20.0	5.8	17.0	1.0
20C	>30.0	1.7	>20.0	9.5	50.0	6.8
24C	26.0	3.6	>20.0	15.5	45.0	2.0
28C	>30.0	1.4	>20.0	13.5	>100.0	5.8

Salinity Effects on Acute Metals Toxicity.

There was a possible effect of salinity on the toxicity of Cu and Ag to *G. splendens*, but *I. galbana* did not respond similarily to Cu and Ag or Fe, Zn and Hg.

Salinity had an indirect effect on the toxicity of Cu and Ag to *G. splendens*. Toxic effects were progressively less in media with progressively higher salinity levels from 14 ppt to 28 ppt (TABLE 10).

Unlike the toxicity of Cu and Ag at different salinity levels to *G. splendens*, the results of tests with *I. galbana* did not indicate that salinity altered the toxicity of these metals. Also, salinity had no effect on the toxicity of Fe, Zn and Hg to *I. galbana* (TABLE 10). Tests with Cu in media with seven different salinity levels from 7 ppt to 37 ppt had MTL values from 0.13 to 0.15 mg Cu/L except in medium with 37 ppt salinity in which the Cu was less toxic (MTL=0.3 mg Cu/L).

TABLE 10. EFFECTS OF SALINITY ON THE TOXICITY OF METALS TO *G. splendens* AND *I. galbana* (VALUES IN MG/L)

	G. splendens							
	M-50	MTL	M-50	MTL	M-50	MTL	M-50	MTL
	Ag-20C		Cu-30C					
14 ppt	.021	.0013	.043	<0.005				
16 ppt	.024	.0065	.062	<0.005				
20 ppt	.030	.0085	>0.10	0.01				
24 ppt	.052	.0060						
28 ppt	.047	.0094	>0.10	0.034				
	I. galbana							
	Cu-20C		Ag-20C		Fe-20C		Zn-20C	
7 ppt	>1.0	.13			9.0	1.55	6.8	4.4
10 ppt	>1.0	.15	.09	.025				
14 ppt	>1.0	.14	.08	.025				
16 ppt	>1.0	.14						
20 ppt	>1.0	.15	.086	.024	8.5	1.6	3.2	2.7
28 ppt	>1.0	.13	.081	.026				
37 ppt	>1.0	.30			9.8	1.45	3.8	2.3
	Hg-20C							
12 ppt	.18	.11						
20 ppt	.16	.10						
28 ppt	.17	.10						

Combined Effects of Temperature and Salinity on Metals Toxicity.

The effects of temperatures near the tolerance limits (14 and 30C) in combination with salinity levels of 14 ppt and 28 ppt were employed to evaluate the effects of temperature and salinity combined on the acute toxicity of metals to *G. splendens*. In addition, tests with Ag, Cu and Hg were conducted at 32C and 33C at 14 and 28 ppt salinity.

The interacting effects of temperature and salinity on metals toxicity did not follow a specific toxicity trend. With the exception of Fe, either the M-50 values, the MTL values or both indicated that the toxic levels of the metals were lower (the toxicity was higher) in media with 14 ppt than with 28 ppt salinity (TABLE 11). Also, values for Mo, Ni, Zn and Cr indicated that these metals were more toxic in medium with low salinity and high temperature (14 ppt and 30C) than in media with other temperature and salinity combinations. Although the M-50 and MTL values did not show that V, Ag, Cu and Hg were more toxic in low salinity medium at high temperatures, compared to the levels with high salinity and low temperature conditions, the toxicity levels of these metals with these conditions were low. The control test populations in conditions of 33C and 14 ppt salinity died within 48 hours whereas those in portions subjected to 33C and 28 ppt grew at a slow rate. The Fe-MTL values were higher at 30C than at 16C in both 14 ppt and 28 ppt salinity media. Also, this was true for the M-50 values. The toxicity of Fe to *G. splendens* in media with these four temperature and salinity combinations were the only results that indicated that temperature had increased toxicity more than salinity. The lowest toxicity levels (highest MTL and M-50 values) were at 30C rather than at 16C.

The effects of different combinations of temperature and salinity on the toxicity of Zn, Hg, Ag and Cu to *I. galbana* were not significantly different. High and low MTL and M-50 values associated with some T-S combinations were not significantly different from other results with the same or similar temperature-salinity, metal and organism combinations (TABLE 12). These combinations were either similar or within range of the LMTL and HMTL for *I. galbana* and these four metals (TABLES 2, 3 and 5). Some organisms (<50%) survived for 48 hours at 30C, but not if the portion had .02 µg Cu/L or more added at this temperature in 14 ppt salinity medium.

Results of tests on the combined effects of temperature and salinity on metals toxicity to *T. pseudonana* (Clone 3H) indicated that the metals were more toxic at 14 ppt than at 28 ppt. The MTL and M-50 values for Cr and Ni and the Mo-MTL values did not necessarily follow this trend (TABLE 13). Tests results with the other six metals ususally indicated that the metals were more toxic with low salinity-high temperature conditions.

The M-50 value of Mo in 14 ppt salinity medium was less than 100 mg/L at the 5 temperatures employed and more than 100 mg/L with 28 ppt salinity medium (TABLE 13). This difference did not occur in the MTL value, but 4 of 5 MTL values in 28 ppt medium were more than 10.0 mgMo/L, whereas 3 of 5 values at 14 ppt were less than 10.0 mgMo/L. The mean M-50 value for Mo at 14 ppt and 28C was the lowest M-50 value for this metal.

TABLE 11. COMBINED EFFECTS OF TEMPERATURE AND SALINITY ON THE TOXICITY OF METALS TO *G. splendens* (VALUES IN MG/L)

	Ag				Cu				Hg			
	M-50				M-50				M-50			
	16C	30C	32C	33C	16C	30C	32C	33C	16C	30C	32C	33C
14 ppt	.018	.021	.020	CD[1]	.030	.028	.045	CD	.018	.016	.025	CD
28 ppt	.033	.044	.025	.015	.087	.057	.050	.008	.06	.074	.09	.015
	MTL				MTL				MTL			
14 ppt	.007	.011	.002	CD	.019	.010	NG[2]	CD	.008	.004	.005	CD
28 ppt	.008	.010	.002	.01	.038	.018	.028	.004	.011	.014	.010	.005

	Cr		Ni		Mo		Fe	
	M-50		M-50		M-50		M-50	
	16C	30C	16C	30C	16C	30C	16C	30C
14 ppt	.3	.25	8	6.5	4.5	6	1.0	1.1
28 ppt	.22	.24	20	38	16	13	0.9	3.7
	MTL		MTL		MTL		MTL	
14 ppt	.026	.012	1.8	0.4	1.6	1.3	.15	.28
28 ppt	.028	.022	2.5	1.8	2.4	5.2	.16	.28

	Zn		V	
	M-50		M-50	
	16C	30C	16C	30C
14 ppt	2.3	6.0	1.8	7
28 ppt	6.1	3.5	24	42
	MTL		MTL	
14 ppt	.25	1.4	.64	2.2
28 ppt	.7	.3	11	26

[1] CD = Controls died

[2] NG = No growth

TABLE 12. EFFECTS OF TEMPERATURE-SALINITY COMBINATIONS ON THE TOXICITY OF Zn, Hg, Ag and Cu TO *I. galbana*

	Zn		Hg		Ag		Cu	
	M-50		M-50		M-50		M-50	
Temperature C	16C	28C	16C	28C	16C	28C	16C	28C
Salinity ppt								
12	3.4	1.0	0.28	0.20	0.08	0.096	0.68	0.11
16	2.8	3.0	0.33	0.18	0.011	0.11	0.76	0.22
20	2.4	0.8	0.31	0.28	0.21	0.14	0.85	0.35
28	2.4	3.0	0.3	0.09	0.33	0.55	0.55	0.54
	MTL		MTL		MTL		MTL	
12	2.0	1.0	1.3	.10	0.022	0.015	0.035	0.01
14								
16	0.43	0.36	0.074	0.058	0.024	0.038	0.02	0.07
20	0.81	0.23	0.1	0.12	0.064	0.060	0.07	0.05
24								
28	1.2	0.43	0.1	0.11	0.024	0.11	0.018	0.025

TABLE 13. COMBINED EFFECTS OF TEMPERATURE AND SALINITY ON THE TOXICITY OF METALS TO *T. pseudonana* (CLONE 3H) (VALUES IN MG/L)

Temperature C	12C	16C	20C	24C	28C	12C	16C	20C	24C	28C
Salinity ppt		Mo M-50						Zn M-50		
14 ppt	90	64	66	70	52	0.5	2.8	1.8	.75	2.0
28 ppt	>100	>100	>100	>100	>100	>20	>20	>20	>10	>20
		MTL						MTL		
14 ppt	60	3.5	35	8.6	4.0	<0.1	.17	<.1	<.1	.2
28 ppt	15	89	80	<1.0	30	.35	.28	.1	<.1	.17
		Fe M-50						Cr M-50		
14 ppt	>20	15	15	15	15	>1	>1	.35	.48	.60
28 ppt	19	>20	>20	>20	>20	>1	>1	>1	>1	>1
		MTL						MTL		
14 ppt	12	11.5	11	12	12	.065	.058	.028	.017	>.01
28 ppt	12.5	9.7	>20	>20	>20	.062	.037	.046	.082	.15
		V M-50						Hg M-50		
14 ppt	26	18	13	12	21	.048	.043	.056	.049	.030
28 ppt	35	27	35	33	37	.088	>.1	.095	.075	.073
		MTL						MTL		
14 ppt	12	9.8	8.4	7.9	8.6	.027	.031	.032	.034	.018
28 ppt	19	19	23	20.0	16.0	.039	.053	.062	.047	.043
		Ni M-50						Cu M-50		
14 ppt	>10	>10	0.4	0.1	>10	.06	.12	.19	.09	.07
28 ppt	>10	>10	0.9	.18	>10	.64	.71	.56	.19	.71
		MTL						MTL		
14 ppt	.10	.031	.028	.017	.08	.012	.046	.048	.031	.017
28 ppt	.072	.14	.030	.021	.18	.082	.068	.052	.072	.034
		M-50			Ag		MTL			
3 ppt	.025		.20		.028	.014		.084		.013
5 ppt	.074		.16		.047	.03		.07		.016
7 ppt	.046		.13		.027	.023		.068		.014

(continued)

TABLE 13 (continued)

Temperature C	12C	16C	20C	24C	28C	12C	16C	20C	24C	28C
Salinity ppt										
					Ag					
		M-50						MTL		
10 ppt	.071	.14	.19	.075	.038	.031	.074	.076	.044	.021
14 ppt	.095		.16		.056	.037		.056		.032
21 ppt	.052		.12		.063	.023		.068		.040
28 ppt	.07	.19	.15	.080	.088	.025	.031	.042	.048	.052

There were no significant effects of temperature on the toxicity of Fe and Zn at 14 and 27 ppt. As previously noted, Fe additions as high as 10 mg Fe/L stimulated the growth of *T. pseudonana*.

Although 7 of 10 Cr M-50 values were more than 1.0 mg/L (the maximum added), the three Cr M-50 values that were less than this concentration were in 14 ppt salinity media. On the other hand, Cr-MTL values indicated that Cr was more toxic at 14 ppt than at 28 ppt salinity in combination with 24C and 28C.

Vanadium was more toxic to *T. pseudonana* at 14 ppt than in medium with 28 ppt salinity. Although portions of *T. pseudonana* cultures that were in 14 ppt medium had lower MTL values than those in 28 ppt, high concentrations of V were tolerated by *T. pseudonana* and there were no combinations of temperature and salinity that uniquely elicited a higher or lower toxic level of this metal.

The toxicity of Hg and Cu to *T. pseudonana* was less at 28 ppt than at 14 ppt salinity. Also, with the exception of comparable M-50 and MTL values at 12C and 14 ppt salinity, these toxicity levels were less in conditions of 28C and 14 ppt than with any other combination. High and low temperatures combined with 14 ppt salinity had significant effects on the toxicity of Hg and Cu to *T. pseudonana*. Hg and Cu were more toxic at 12C and 28C than at 16, 20 and 24C.

The toxicity of Ag to *T. pseudonana* was tested at seven salinity levels between 3 ppt and 28 ppt in combination with either three or five temperature levels (TABLE 13). The Ag M-50 and MTL values were higher at 20C than at either 12C or 28C at all salinity levels. The M-50 values for Ag at 3 ppt were among the lowest of the values determined.

The Ag MTL values were higher at 20C than at 12C or 28C. Also, except for those values in combination with 21 ppt and 28 ppt salinity the MTL values at 12C were higher than those at 28C. The Ag MTL value (0.013 mg Ag/L) that occurred with the highest temperature (28C) and lowest salinity (3 ppt) was the lowest value (highest toxicity) recorded in assays with *T. pseudonana*, but it was comparable to the Ag MTL (0.014 mg Ag/L) recorded for two other T-S combinations (3 ppt, 12C; 7 ppt, 28C). The Ag MTL values with 28C increased as the salinity was increased. The Ag MTL values in medium with 28 ppt increased (less toxic) as the temperature level was increased from 12C to 28C, but this apparent combined effect of temperature on toxicity at the other salinity levels employed did not occur.

SECTION 5

INSTANTANEOUS EFFECTS OF METALS ON PHOTOSYNTHESIS RATES OF *G. splendens, T. pseudonana* AND *I. galbana*

Much of the work pertaining to the toxicity of metals to phytoplankton has been conducted in freshwater and at concentration levels aimed at controlling or eliminating the algal populations. Results indicated that the toxicity of copper varied considerably from one time to another and depended on temperature and alkalinity (Bartsch, 1954). The composition of saltwater would probably make a more complex condition with respect to reactions with metals. Erickson, Lackie and Maloney (1970) determined that there was a wide variation in the tolerance between different members of the estuarine phytoplankton community to metals.

MATERIALS AND METHODS

Bacteria-free, unialgal cultures of *I. galbana, G. splendens* and *T. pseudonana* were grown in a chelate-free artificial seawater medium (TABLE 1) with the major salts (NaCl, KCl, $MgSO_4$, $MgCl_2$ and $CaCl_2$) adjusted to result in water with a salinity level of 20 ppt. Medium was prepared with triple glass distilled water. Glassware was cleaned as previously described (Aldrich and Wilson, 1960). Photosynthesis was measured by using a modified method of Steeman-Nielson (1952) and Strickland and Parsons (1968). A Coulter Counter Model B was used for cell counts and radioactivity was counted with a Nuclear Chicago Model 4338B counting system. The organism to be used in an experiment was growing in a batch culture. Triplicate, 100 ml portions of the culture were placed in 125 ml Fernbach Flasks which received 1 μc ^{14}C, incubated for 1 to 24 hours at 21 to 25C in 800-1000 ft. C of "cool white" fluorescent light. After incubation, the photosyntheses rate was measured by withdrawing a culture portion and carry out the described procedure.

The remaining culture portion of each flask received a specific volume of a metal solution to give the desired concentration of the metal to be tested. Triplicate 10 ml amounts of each metal concentration were dispensed into 16 x 125 mm test tubes furnished with a polypropylene closure. The culture portions were incubated for 24 hours and filtered on a 0.45 μ membrane and the ^{14}C uptake was counted. All test tubes were maintained at a uniform distance from the light during incubation.

RESULTS

Although Cu concentrations as low as 10 μg Cu/L depressed both the short-term photosynthesis and growth rate of *I. galbana,* concentrations as high as 1250 μg Cu/L did not stop cell division or photosynthesis. The doubling time increased from 32 hours in the control to 62 hours in medium with 62.5 μg Cu/L and up to 224 hours in medium that received 1250 μg Cu/L. Culture poritons that received 1250 μg Cu/L had a photosynthesis rate that was 24% of the control (no metal added) after 120 hours exposure.

G. splendens grew less in medium that received 70 or 80 μg Cu/L after incubation periods up to 24 hours than in the control medium, but recovered and had increased growth after periods of 48 hours or more. Addition of 20 μg Cu/L had no effect on the growth of this organism.

Culture portions of *I. galbana* were exposed to medium with Cr additions of 1 to 8 mg Cr/L. Additions within this range reduced the photosynthetic activity compared to the control (no metal added), but after 96 hours exposure the culture portions recovered and organism from all portions grew after being transferred to fresh NH-15-CF medium. Also, the color of organisms in portions that received 8 mg Cr/L was green rather than the normal yellow-brown.

I. galbana culture portions that received additions of 20 μg Hg/L had a mean photosynthesis rate that was 58% higher than the mean control portion. The former (20 μg Hg/L) portion had a mean cell number after 48 hours incubation that was 33% higher than the control portions. Hg reduced photosynthesis of *I. galbana* more than comparable Cu additions. Also there was a 16% increase of cell numbers after 48 hours in portions that received 160 μg Cu/L.

The growth rate of *T. pseudonana* was modified by Hg additions. Growth was not uniform in respect to Hg amounts added. Culture portions exposed to 40 μg Hg/L increased in cell numbers less than 10% after 96 hours, but culture portions that received 10 μg Hg/L or less (control portions) increased approximately 400%. Medium portions that received 20 and 40 μg Hg/L grew much less than the control.

Additions of 1.0 mg Hg/L stopped cell division of *I. galbana* and it did not resume within 72 hours. Culture portions that received additions of 70 μg Hg/L had a growth rate that was reduced by 47% after 1 hour compared to the controls. With additions of this level the photosynthesis rate of *I. galbana* recovered to its pre-test condition 4 hours after the addition was made. The ^{14}C uptake rate was reduced after 2 hours exposure to medium with an addition of 80 μg Hg/L. In this same experiment, additions of 80 μg Hg/L reduced ^{14}C uptake within the same 2 hour period, but after 72 hours, the lowest addition tested (5 μg Hg/L) reduced the ^{14}C rate to levels as low as 89% of the control. Carbon-14 uptake was less in medium with additions of 40 and 80 μg Hg/L, but the cells continued to divide in these portions. Lower addition of Hg (5 and 10 μgHg/L) had higher ^{14}C uptake rates than the controls after 96 hours.

SECTION 6

THE EFFECTS OF LIGHT INTENSITY, TEMPERATURE, CELL DENSITY AND CHRONIC EXPOSURE OF MERCURY AND CHROMIUM ON TWO MARINE PHYTOPLANKTERS IN CONTINUOUS CULTURE

The concentration at which some metals promote growth is usually only slightly less than the concentration at which they are toxic. Copper has been reported to be essential for the synthesis of cytochromes, peroxides, catalases and it has been demonstrated that Cu is necessary for the development of chlorophyll (Corcoran and Alexander 1964), yet it has been reported to be an algal toxicant, e.g. Marvin *et al*, 1960; Steemann-Nielsen and Wium-Anderson 1970; Niemi 1972. Sverdrup *et al*, (1942) reported an average of 10 μg Cu per liter in natural seawater and Guillard and Ryther (1962) added 5 μg Cu per liter when enriching seawater. Wilson (1958) found 30 μg Cu per liter was lethal to *Gymnodinium breve* and Mandelli (1969) found the growth of three species of marine flagellates was inhibited by copper concentrations as low as 30-50 μg per liter. These combined results indicate that the inhibitory concentration of copper is only two or three times the concentration that supports phytoplankton growth.

Johnston (1964) suggested that the species composition of a phytoplankton community as well as overall phytoplankton growth was associated with the physical and/or chemical availability of trace metals. A phytoplankton population may be limited by the availability of a trace metal as follows: (1) standing crop--the total biomass attainable by a population of algae may be limited by the total amount of a metal present (biologically non-active reservoir); and (2) productivity--the growth rate may be limited by the flow rate of a metal (replenishment rate). The effect of a trace metal upon phytoplankton growth must be related in some manner to the capability for uptake and retention of the metal. For example, Spencer (1957) demonstrated the growth-promoting activity of manganese is related to the concentration of the ionic form, and is independent of the non-ionic concentration. Thus, the chemical form in which a metal is present may control its biological availability, and the flow rate of a metal may be a consequence of the rate at which the biologically active form is replenished from the non-active reservoir. Provasoli (1958) suggested: "By balancing chelators and trace metal concentrations one can create a non-toxic reservoir of metals, thus allowing prolonged division of fast-growing organisms".

Wilson and Collier (1955) and subsequently, Johnston (1964), Barber and Ryther (1969), and Prakash and Rashid (1968) have shown that the addition of known chelators or natural humic substances will enhance the growth of phytoplankton in seawater. Traditional interpretation has suggested that chelation makes trace elements available for biological uptake, but Steeman-

Nielsen and Wium-Anderson (1970) argue that an inhibiting concentration (ionic) of a metal, principally copper, could be rendered non-toxic by chelation. Except for the study of Wilson (1966) there are few studies on complexed metal ions accompanying growth promotion studies of phytoplankton. Slowey *et al* (1967) and Alexander and Corcoran (1967) have demonstrated that up to 50% of the copper in seawater may be complexed into an organic form. Fitzgerald and Lyons (1973) and Knauer and Martin (1972) indicated a similar percentage of Hg in the complexed form. Thus, organic complexes of both mercury and copper may constitute at least 50% of their total concentration, if a chelator is present.

Data obtained from acute bioassays may vary depending on the time lapse between the addition of a metal and analysis of parameters to evaluate the metal's effects. Steemann-Nielsen and Wium-Anderson (1970) found that 20 minutes after adding a toxic concentration of copper to static cultures of a diatom the photosynthesis was reduced 10%, while after 40 minutes photosynthesis was reduced 50% and after 60 minutes there was a 60% reduction in photosynthesis. Studies allowing longer exposure times prior to determining the effects on growth rates may be further complicated by an extension of the lag phase before cell division starts. Ben-Bassat *et al* (1972) showed the lag phase of *Chlamydomonas* was extended when exposed to increasing concentrations of mercuric chloride. After cell division started, the growth rate was similar to that of the control and the final population level approached that of the control. Thus, the time factor is of great importance in a biossay of this type, and comparison of different studies are complicated by a variety of exposure times considered appropriate by investigators (e.g., Erickson *et al* 1970; Harriss *et al* 1970; Niemi 1972; Nuzzi 1972).

Time related factors to be considered with biologically active metals in seawater are removal of the metal from the medium by surface deposition on container walls or particulate matter, precipitation, and phytoplankton uptake. Duursma and Sevenhuysen (1966) found that soluble concentrations of metals are normally reduced in seawater by precipitation into insoluble compounds, usually hydroxides or carbonates, which have low solubility constants. They determined that mercury (added as $HgCl_2$) in Pyrex flasks of filtered seawater, decreased 50% in 2 hours and after 6 hours the initial concentration was reduced by 90%. The loss of Hg from solution was attributed to surface deposition, in that the Hg was recoverable with acid treatment of the flask walls (Rice *et al* 1973). Mandelli (1969) reported a rapid uptake of copper by five species of marine phytoplankton during the first 15-30 minutes of exposure to various concentrations of copper and the total uptake was related to the initial concentration of the metal. A similar rapid uptake of Hg^{203} by a diatom was reported by Gloosehenko (1969) and subsequent uptake depended upon continued cell division.

The dynamic (continuous culture) bioassay is best suited for determining the chronic effects of a test material at acutely sub-lethal concentrations. In a dynamic culture the time factor, both in relation to the population in the culture and in relation to the metal concentration, is reduced or eliminated. After steady-state growth of the test organism is established, the culture is time independent and its functions are not functions of time; thus, one need not be concerned with the age of the culture (Malek 1966).

The essential feature of this technique is that growth in a dynamic culture takes place under steady-state conditions; that is, growth occurred in a constant environment and at a constant rate.

The distinct advantage of a continuous culture system is the capacity to continuously renew a predetermined concentration of the test material in the culture regime. As previously discussed, metals in solution are normally reduced in seawater by precipitation, absorption and phytoplankton uptake. In a continuous culture system the ionic form of a metal and the medium are added to the culture vessel through separate inflow tubes simultaneously. The metal flow rate is maintained as a constant ratio of the medium flow rate. Thus, specific concentrations of a metal ion can be maintained in steady-state equilibrium within the culture, relatively independent of other cations in the medium.

The principal objectives of this study were as follows:

1. The first objective was to determine the effects of Hg and Cr on the growth of *I. galbana* and *G. halli*. Up to 24 concentrations of each metal were established in steady-state cultures ranging from that concentration at which an effect on the growth rate of *I. galbana* and *G. halli* was not detectable to a concentration at which cell division was completely inhibited.

2. The effects of Hg on the growth of *G. halli* in cultures that were growing with a combination of sub-optimal irradiance and temperature was evaluated.

MATERIALS AND METHODS

Continuous Culture Apparatus

The continuous culture apparatus employed for this study was of the turbidometric type (Freeberg 1971). The basic design (Figure 3) consisted of a 1-liter round bottom culture flask housed in a waterbath box, in which water was continuously circulated. The waterbath temperature was maintained (± 0.6C) by a Dapper & Company Model A4406B Mobile force flow unit which resulted in a constant culture temperature (± 0.2C). A silicon (P-N junction type) photocell was housed in each water-jacketed box on opposite sides of the waterbath box, and a monitoring lamp was positioned to maintain equal illumination on both photocells. The photocells were connected in series and the microampere output difference between the two photocells was amplified and metered in a control center. If the metered microampere signal exceeded a preset value, a solenoid was activated which allowed the inflow of fresh medium into the culture vessel until the signal decreased below the preset value. Thus, the population density, represented by turbidity between the photocells, was held constant (± 5%).

The control center amplification unit was modified by replacing the 1.5 volt battery, of the low voltage circuit, with a variable microampere DC power supply. The power supply could be regulated to provide the amperage necessary to nullify any difference, within limits, between the microampere

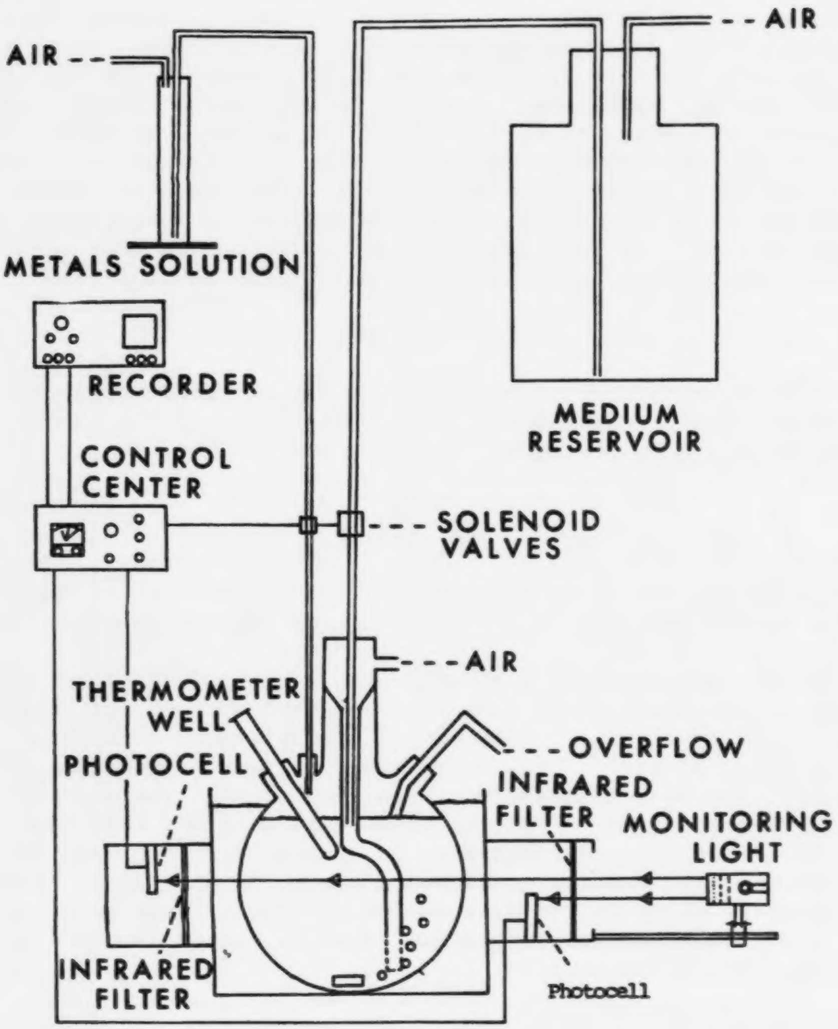
AIR
AIR
METALS SOLUTION
RECORDER
MEDIUM RESERVOIR
CONTROL CENTER
SOLENOID VALVES
AIR
THERMOMETER WELL
PHOTOCELL
OVERFLOW
INFRARED FILTER
MONITORING LIGHT
INFRARED FILTER
Photocell

Figure 3. Diagrammatic sketch of the basic turbidostat design.

output of the two photocells. Thus, a range of population levels (turbidity) could be selected, at which a culture was maintained in the turbidostat.

A delivery system capable of supplying metallic ions in solution to the culture vessel simultaneously with the medium, yet independent of the medium, was developed for the turbidostat (Figure 3). A 100 milliliter graduated cylinder was connected to the culture vessel by small diameter silicone tubing. The addition of metal solution was controlled by a solenoid, which operated in a similar manner to that of the medium supply system. The two solenoid pinch valves were activated simultaneously by the control center, and the metal solutions flow rate was 1, 2 or 3% of the medium flow rate. The relative flow rates of the solution and medium were established by adjusting the orifices at the tips of the glass inlet tubes. The amount of metal solution (M_V) delivered to the culture vessel was determined by subtracting the volume prior to the test and the theoretical metal concentration (Cm) in the culture vessel during steady-state conditions was calculated as follows:

$$M_A = M_V \times M_C$$

M_A is the total amount of metal ion added to the culture vessel, M_V is the volume of metal solution delivered to the culture vessel, M_C is the concentration of the metal ion in the metal solution; and

$$C_m = \frac{M_A}{dV}$$

where C_m is the theoretical metal concentration in the culture vessel during steady-state conditions, and dV is the volume of culture overflow.

The energy necessary for photosynthesis was supplied by irradiance from two 150-watt incandescent flood lamps--one on each side of the waterbath. The spectral quality of the irradiance was controlled by a chemical filter which was an integral part of the waterbath box. Jerlov (1951) classified various coastal and oceanic seawater types according to their transparency to daylight. The spectrum of the filtered tungsten light more nearly simulated that of Jerlov's coastal seawater No. 2 than either of the two commonly used fluorescent light sources (cool-white and white daylight). Light intensity was varied by changing the distance of the flood lamps from the culture vessel and the irradiance was determined in energy units (mw/cm^2) with a YSI-Kettering Model 65 Radiometer.

Culture Methods and Materials

The two species of marine phytoplankton employed in this study were *Isochrysis galbana* Parke (Chrysophyta), a small (5-6 μm diameter) naked flagellate; and *Glenodimium halli* Freudenthal and Lee (Pyrrophyta), a lightly armored dinoflagellate. Axenic cultures of these organisms were maintained at the Texas A&M University, Department of Marine Sciences Laboratory in Galveston, Texas. The culture of *I. galbana* was isolated by M. W. Parke from the saltwater fish ponds of Port Erin in 1938 and obtained by the Texas A&M laboratory from Dr. R. Ukeles, National Oceanic and Atmospheric Administration (National Marine Fisheries Services) Laboratory, Milford, Connecticut.

The *G. halli* culture was isolated from Offats Bayou, Galveston in 1955 by W. B. Wilson (personal communication). These two species were employed in this study because each species could tolerate the mechanical stirring required to maintain homogeneous distribution without cell damage or attachment to the culture vessel, and both species have a specific growth rate of approximately 1.0 div/day. The growth requirements of *I. galbana* have been studied and it has been commonly employed in phytoplankton investigations (e.g., Kain and Fogg, 1958; McLaughlin, 1958; Caperon, 1968; Davies, 1974). The growth requirements of *G. halli* have not been determined and culture studies employing *G. halli* at this time, other than in this laboratory, are unknown.

The artificial seawater media employed was basic formulation from Gates and Wilson (1960) and was modified by W. B. Wilson (personal communication) as shown in TABLE 1 for this study.

Experimental metals solutions were made by dissolving the chloride salts of Hg^{+2} and Cr^{+3} in glass distilled water. Stock solutions of 100 μg Hg or Cr per ml were prepared in 500 ml glass stoppered volumetric flasks (67.7 mg $HgCl_2$ or 128.2 mg $CrCl_3 \cdot 6H_2O$ per 500 ml water). Solutions were made by distilled water dilution of the stock solution. The metal stock solutions and all dilutions were autoclaved at 15 psi for 15 minutes. Aseptic manipulation of stock solutions decreased the possibility of contamination.

All glassware and silicone tubing that was in contact with the medium, metal solutions or the culture were cleaned using the procedure of Aldrich and Wilson (1960). Glassware and tubing were placed in, or filled with, a detergent (Alconox--approximately 5 g/liter) and heated to near boiling (90C) for 15 minutes. The detergent was removed by rinsing with distilled water and brushing the glassware surfaces. The glassware and tubing were placed in or refilled with distilled water, heated to 100C, and then rinsed with distilled water. The glassware was filled with 10% (V/V) HNO_3, heated to 50-60C, emptied, and rinsed with distilled water eight times. The culture vessel was filled with glass distilled water and pretreated by autoclaving at 15 psi for a minimum of 20 minutes.

Upon completion of the cleaning procedure, the culture vessel assembly, tubing, overflow catch vessel, and two cotton air filters were autoclaved at 15 psi for a minimum of 15 minutes. The culture system was assembled while positive sterile air pressure was maintained in all containers that required aseptic conditions. This procedure decreased the probability of bacterial contamination during assembly, inoculation, and normal operation. The air for aeration and positive pressure was supplied by an Aquarium Pump Supply Co. Silent Giant, Model 120 air pump. The air was filtered by a Koby Inc. Air Purifier and Flow Equalizer, Model, Junior, a sterile cotton filter, and connected to the culture air inflow with Tygon tubing. All joints were flamed during assembly. After the culture apparatus was assembled, the culture vessel was filled with medium and the inoculum was introduced aseptically into the vessel through the thermometer well port.

A magnetic stirrer with a teflon-coated stirring rod in the culture vessel was employed to facilitate homogeneous mixing. Failure to maintain a homogeneous population can lead to erratic oscillations in the population density (Powell, 1955). If the magnetic stirrer was stopped, such oscillations were detected by significant differences in the cell counts of samples within a period of less than 24 hours.

Bacterial sterility tests were conducted biweekly, or if contamination was suspected. An unexpected decrease in growth rate or the attachment of cells to the vessel walls usually indicated bacterial contamination. Sterility tests were made by aseptically transferring 1.0 ml of culture into each of two media. One medium contained 10 ml of peptone seawater broth, the other a 10 ml peptone seawater agar slant (Spencer 1952). Tests were incubated at 37C for 10 days.

The cell numbers of a continuous culture was considered to be a variable factor affecting the physiology of the organism in culture. The flow of medium or test material per cell (flux) may change with different cell numbers. This hypothesis was investigated by determining the K_c, Chl *a*, and ^{14}C uptake/cell of each species over a wide range of population levels in steady-state growth at 23C with irradiance levels of 5, 10 and 15 mw/cm^2. A standard cell concentration for each species was employed in all tests. The standard population level selection was the minimum level at which the turbidostat control center was able to maintain the population level constant within a ± 5% range; and the concentration at which the least change in K_c and Chl *a*/cell occurred within that range.

Irradiation intensities of 5, 10 and 15 milliwatts per square centimeter (mw/cm^2), which represented approximately 14, 29 and 43% of the energy available in full sunlight, were supplied to the cultures. Eppley *et al* (1969) reported full sunlight measured in energy units was equal to approximately 35 mw/cm^2 (1 langly/min = 70 mw/cm^2). The three intensities simulated the energy that would be expected at depths of 8.2, 4.7 and 2.3 m, respectively, in water classified by Jerlov as coastal seawater No. 2 (Jerlov 1954). Ryther (1956) suggested the factor, 70 mw/cm^2, equals 15,500 ft-c, for converting the photosynthetically active irradiation of full sunlight (400-700 mw/m^2) to units of illumination. Therefore the irradiation levels employed gave theoretical illumination values of approximately 1100, 2200 and 3300 ft-c which were adequate for light saturation. Illumination values of 700, 1400 and 2100 ft-c were determined with a Gossen Luna Pro exposure meter. The Luna Pro meter could be read only to the nearest 100 ft-c in the range of 1000 to 5000 ft-c.

The interrelationship of light and temperature on the growth of each species was investigated by varying the temperature of three turbidostats simultaneously in 3C increments or, in a few instances, 2C increments. The temperature was varied from 14 to 28C with *I. galbana* and from 20 to 35C with *G. halli*.

The K_c, Chl *a*/cell and ^{14}C uptake/cell were determined for cultures of each organism with steady-state concentrations of $HgCl_2$ and $CrCl_3$. The metal concentrations in the culture were changed by varying the concentration of the metallic salt solution. Concentrations of each metal were varied within a range from a minimum, at which the K_c was not significantly affected, either beneficially or deleteriously, to a maximum at which cell division was inhibited and the K_c approached zero. The K_c, Chl *a*/cell and ^{14}C/cell uptake were determined in control cultures of each organism, in which 1, 2 or 3% distilled water were delivered in place of the metals solution.

Chlorophyll *a* was determined daily using a modification of Strickland and Parson (1968). Ten-ml duplicate portions were removed from a 40-ml culture sample. The remaining 20-ml portion was used for cell enumeration with a Model B Coulter Counter. Each of the two chlorophyll *a* samples were filtered on a Whatman GF/C type filter pad that had received 0.5 ml of a distilled water 1% $MgCO_3$ solution (Yentsch and Menzel, 1963; Long and Cooke, 1971). The filter pad was extracted with 90% acetone, ground with a Thomas Tissue Grinder #B51590, centrifuged and the fluorescence of the supernatent was measured with a Turner Model 111 Fluorometer as modified by Lorenzen (1966). Cells enumerated with a Model B Coulter Counter are correct to within 2% (Mattern *et al* 1957; Caperon 1968).

The specific growth rates of the populations were calculated as follows:

$$K_n = \frac{1}{n}\frac{dn}{dt},$$

n is the number of organisms per unit volume and t is time (Novick and Szilard 1950). Assuming the number of organisms per unit volume was constant, the specific growth rate may be determined by

$$K_v = \frac{1}{V}\frac{dv}{dt},$$

if V is the volume of the culture. The turbidostats employed were capable of maintaining the population number constant within ± 2% with ideal conditions. This capability did not normally occur because of (1) sampling errors and accuracy of cell enumeration; (2) the position of the monitoring light was not stable (mounting of lamp housing), thus the light path changed; and (3) changes in optical density between the two photocells which was not due to the culture, e.g., condensation on windows, air bubbles on the side of the culture vessel, and waterbath turbidity. The population level was normally maintained within ± 5%. The assumption of constant population number was validated by mathematically correcting the overflow volume by the percent population level change as follows:

$$V_c = \frac{n_2-n_1}{\frac{n_1+n_2}{2}} \times V,$$

where V is the total volume of the culture. The volume correction (V_c) was added to the overflow volume and K_c was calculated as follows:

$$K_c = \frac{1}{V}\frac{d_V+V_c}{dt}$$

The culture overflow was measured and K_c calculated twice each day. The K_c

values are volume of constant culture overflow per volume of the culture vessel per day which, if the chlorophyll *a*/cell remains, are directly related to divisions per cell per day.

The photosynthetic response of each species to changes in physical environment and various concentrations of mercury and chromium were evaluated from daily radioactive carbon/cell uptake. A 50 ml portion was obtained from the culture vessel and triplicate 10 ml portions were placed into screw-cap test tubes. The remaining portion was used for cell enumeration. One ml of ^{14}C-labeled $NaHCO_3$ solution containing 1 microcurie of activity was added to each of the 3 test samples. The samples were incubated in the corresponding turbidostat waterbath box for 1 hour at the same temperature and irradiation as the steady-state culture. The cells were concentrated on glass fiber filters (type GF/A) dried for a minimum of 12 hours and the radio activity was counted 3 times for 1 minute.

RESULTS

Culture Conditions--*Isochrysis galbana*

The specific growth rate (K_c) of *I. galbana* was inversely related to the cell concentration (TABLE 14). In cultures with the lowest population level tested, 50,000 cells/ml, there was a maximum K_c of 1.57 and the K_c decreased with population levels greater than 200,000 cells/ml to a minimum K_c of 0.45 with 400,000 cells/ml. The K_c was constant (1.16 to 1.17 ± 0.13) with cell numbers of approximately 75,000 to 200,000 cells/ml. The K_c determined in two different turbidostats, one exposed to 5 mw/cm^2 and the other exposed to 10 mw/cm^2 irradiation, were similar at each of the population levels tested, with one exception. With cell numbers of 200,000 cells/ml, the K_c of the culture irradiated with 10 mw/cm^2 (1.17) was 27% greater than the culture with 5 mw/cm^2 (0.92). The medium flow rate/cell/day (flux) decreased as the cell concentration increased. There was a maximum flux of 31.4 x 10^{-6} ml/cell with 400,000 cells/ml.

The chlorophyll *a* per cell (10^{-8} μg Chl *a*/cell) of *I. galbana* grown at 23C with 5 mw/cm^2 irradiation was higher if cell concentrations were greater than 135,000 cells/ml than in cultures with lesser population levels (TABLE 14). Excluding the apparent anomalous value with population levels of 75,000 cells/ml, the Chl *a*/cell did not change (mean value of 6.54) from the minimum population level tested to approximately 135,000 cells/ml and increased to a maximum of 13.00 x 10^{-8} μg Chl *a*/cell with 300,000 cells/ml (TABLE 14).

The optimum temperature range for the growth of *I. galbana* was from 20 to 25C with the three light intensities employed (TABLE 15). The K_c increased linearly with a slope of 0.065 from 17 to 25C, remained constant at 25C and decreased at temperatures greater than 25C (TABLE 15). *I. galbana* did not grow and the cell concentration of the culture decreased rapidly at 28C with each of the three light intensities tested. The Chl *a*/cell was 26% less at temperatures of 17C than at 20 to 26C and the Chl *a*/cell values were similar from 20 to 26C for each of the three light intensities.

TABLE 14. SPECIFIC GROWTH RATE, FLUX, CHL *a*/CELL AND ^{14}C UPTAKE FOR VARIOUS POPULATION LEVELS OF *I. galbana* GROWN AT 23C AND WITH 5 AND 10 mw/cm^2 IRRADIATION

Irradiation mw/cm^2	Population level cells/ml (X10^5)	Flux[1.] (X10^{-6})	K_c[2.]	Chl *a*/cell µg (X10^{-8})	^{14}C Uptake[3.] (X10^4)
5	.50	27.4	1.37	6.54	2.56
5	.75	15.5	1.16	7.76	2.29
5	1.00	12.0	1.20	6.55	2.91
5	1.25	9.3	1.16	6.24	-
5	1.35	8.7	1.18	6.45	-
5	1.35	8.8	1.19	6.57	4.76
5	1.75	6.7	1.17	7.96	3.79
5	2.00	4.6	0.92	9.29	3.59
5	3.00	2.6	0.79	13.00	1.81
5	4.00	1.1	0.45	11.22	2.37
10	.50	31.4	1.57	-	-
10	1.00	12.2	1.20	5.78	-
10	1.30	9.5	1.21	5.82	-
10	1.30	9.3	1.21	6.30	6.40
10	2.00	6.2	1.17	5.82	2.92
10	3.00	2.3	0.70	-	-
10	4.25	1.2	0.50	-	-

Notes:

1. Flux: The medium flow rate is calculated as follows: $F=\frac{K_c}{PL}X10^6$, where PL is the population level in cells/ml and is given in ml/cell/day.
2. The specific growth rate is calculated as follows: $K_c=\frac{1}{v}\frac{dv+Vc}{dt}$ and is given in divisions per cell per day.
3. The ^{14}C uptake is given as: $\frac{\text{Counts/min}}{\mu\text{g Chl } a}$

TABLE 15. SPECIFIC GROWTH RATE, CHL *a*/CELL, AND ^{14}C UPTAKE OF *I. galbana* EXPOSED TO VARIOUS LIGHT-TEMPERATURE COMBINATIONS

Irradiation mw/cm^2	Temperature °C	K_c[1]	Chl *a*/cell µg (X10^{-8})	^{14}C Uptake[2] (X10^4)
5	17	0.80	4.69	3.72
5	20	1.07	5.44	3.23
5	23	1.16	6.24	-
5	23	1.18	6.45	-
5	23	1.19	6.57	4.76
5	23	1.44	5.52	7.15
5	25	1.21	6.37	2.02
5	25	1.31	8.24	3.89
5	28	0.0	-	-
Mean[3]	-	-	6.10	3.90
10	17	0.79	3.98	4.05
10	20	1.20	4.75	8.76
10	23	1.21	5.82	-
10	23	1.21	6.30	6.40
10	23	1.20	5.78	8.99
10	25	1.22	6.51	3.09
10	28	0.0	-	-
Mean[3]	-	-	5.66	6.40
15	17	0.77	3.09	6.25
15	20	1.13	3.73	12.22
15	23	1.19	4.72	-
15	23	1.23	4.95	6.94
15	23	1.23	4.29	-
15	25	1.14	5.25	5.55
15	25	1.38	5.98	-
15	28	0.0	-	-
Mean[3]	-	-	4.59	6.60

Notes:

1. The specific growth rate is calculated as follows: $K_c = \frac{1}{v}\frac{dv+Vc}{dt}$ and is given in divisions per cell per day.
2. The ^{14}C uptake is given as: $\frac{\text{counts/min}}{\mu g\ \text{Chl}\ a}$ X10^4.
3. The mean value was determined for all temperatures in which cell division occurred, excluding the highest and lowest values.

The Chl *a*/cell was inversely related to the light intensity (TABLE 15). Analysis of variance indicated a reduction of Chl *a*/cell as the light intensity was increased. The mean Chl *a*/cell value for the temperatures with 10 mw/cm^2 irradiation (5.66) was not significantly different from those values with either 5 or 15 mw/cm^2 irradiation (6.10 and 4.59, respectively), but the mean Chl *a*/cell with 15 mw/cm^2 irradiation was 23% less than the mean value with 5 mw/cm^2 irradiation.

The culture conditions designated as standard for *I. galbana* cultures in the remainder of this report are as follows:

1) Population level - $1.2 \pm .10 \times 10^5$ cells/ml
2) Irradiation - 10 mw/cm^2
3) Temperature - 23C

Culture Conditions--*Glenodinium halli*

The K_c of *G. halli* was inversely related to the population level of the culture, if the cell numbers were greater than 25×10^3 cells/ml (TABLE 16). The K_c was constant at 0.98, with a standard deviation of 0.08, from the lowest cell concentration tested (6.8×10^3 cells/ml) to approximately 2.3×10^4 cells/ml with 10 mw/cm^2 irradiation. With 15 mw/cm^2 irradiation, the population level at which the K_c remained essentially constant (0.97 ± 0.09) extended to approximately 6.2×10^4 cells/ml (TABLE 16). The K_c's were similar (0.49 ± 0.04) with 1.2×10^5 cells/ml and 15 mw/cm^2 irradiation.

The medium flux per cell decreased parabolically with increased cell numbers (TABLE 16). Rapid decreases in flux at low cell concentrations corresponded with the plateau region of the K_c curve and as the flux asymtotically approached zero, the K_c leveled off at approximately 0.45. The greatest change of flux corresponded to the maximum slope of the K_c curves.

The Chl *a*/cell did not change significantly with cell numbers at the three light intensities employed. The mean Chl *a*/cell values for 5, 10 and 15 mw/cm^2 irradiation were similar (15.84, 16.49 and 15.68, respectively) (TABLE 16).

Results did not indicate a change in ^{14}C uptake cell with increased cell numbers. The ^{14}C uptake by *G. halli* was higher with higher irradiation levels (TABLE 17). Mean ^{14}C uptake values at 10 and 15 mw/cm^2 (3.38 and 5.06, respectively) were higher than the value with 5 mw/cm^2 irradiation (3.03).

The optimum temperatures for the growth of *G. halli* were from 23 to 32C, with the three light intensities employed in this study (TABLE 17). Mean K_c values increased linearly with a slope of 0.147 from 20 to 29C, remained relatively constant from 29 to 32C, and at 35C, there was no cell division.

Light intensity of 5, 10 and 15 mw/cm^2 had no effect on the mean K_c of *G. halli*. The mean K_c values (0.95, 1.03, 1.24) for all temperatures and each light intensity were not significantly different at the 95% confidence level, although K_c values at some specific temperatures were significantly

TABLE 16. SPECIFIC GROWTH RATE, FLUX, CHL *a*/CELL AND ^{14}C UPTAKE FOR VARIOUS POPULATION LEVELS OF *G. halli* GROWN AT 23C AND WITH 5, 10 mw/cm^2 IRRADIATION

Irradiation mw/cm^2	Population level cells/ml (X10^3)	Flux[1]. (X10^{-6})	K_c[2].	Chl *a*/cell μg (X10^{-8})	^{14}C Uptake[3]. (X10^4)	Medium[4].
5	7.4	118.9	0.88	14.74	-	NH-15Si[5].
5	9.3	98.9	0.92	16.93	-	NH-15Si
5	13.4	64.9	0.87	16.60	3.80	NH-15Si
5	22.7	39.2	0.89	19.16	-	NH-15Si
5	24.4	31.2	0.76	15.93	2.30	NH-15CF
5	28.4	27.1	0.77	15.88	-	NH-15
5	38.0	17.9	0.68	14.84	2.99	NH-15
5	57.4	11.7	0.67	15.99	3.43	NH-15
5	68.0	8.7	0.59	14.37	3.32	NH-15
Mean[6].	-	-	-	15.84	3.25	-
10	6.8	147.1	1.00	17.19	-	NH-15Si
10	7.9	144.3	1.14	18.33	-	NH-15Si
10	9.9	98.9	0.98	15.13	3.55	NH-15Si
10	11.9	82.4	0.98	15.03	-	NH-15Si
10	15.9	38.8	0.95	14.69	-	NH-15Si
10	21.9	44.8	0.98	16.89	-	NH-15Si
10	23.5	39.6	0.93	15.50	3.70	NH-15CF
10	23.8	35.3	0.84	14.93	3.46	NH-15Si
10	32.0	24.4	0.78	14.93	4.90	NH-15Si
10	32.8	23.2	0.76	15.22	4.96	NH-15Si
10	33.4	23.4	0.78	21.88	5.50	NH-15OF
10	36.4	20.9	0.76	-	-	NH-15Si
10	45.5	9.0	0.66	14.26	3.87	NH-15Si
10	57.2	10.0	0.57	15.04	6.59	NH-15
10	61.3	6.9	0.42	18.19	4.02	NH-15Si
10	71.6	5.9	0.42	17.38	3.68	NH-15Si
10	75.5	6.0	0.45	19.04	4.82	NH-15
10	75.9	5.9	0.45	16.91	5.08	NH-15
Mean[6].	-	-	-	16.49	4.41	-
15	6.5	173.9	1.13	15.78	-	NH-15Si
15	13.0	76.9	1.00	14.19	-	NH-15Si
15	20.7	49.3	1.02	21.22	5.50	NH-15CF
15	21.7	41.0	0.89	18.59	4.00	NH-15CF
15	22.5	42.2	0.95	13.89	-	NH-15Si
15	31.0	29.4	0.91	14.78	5.25	NH-15Si
15	47.4	19.8	0.94	15.64	5.41	NH-15
15	61.8	14.2	0.88	15.40	4.54	NH-15
15	119.7	4.4	0.53	15.10	5.58	NH-15
15	151.4	3.2	0.48	16.08	4.74	NH-15
15	196.9	2.3	0.46	15.60	4.16	NH-15
Mean[6].	-	-	-	15.68	4.93	-

(continued)

TABLE 16 (continued)

Notes:

1. Flux: The medium flow rate per cell is calculated as follows: $F = \frac{K_c}{PL} X10^6$ where PL is the population level in cells/ml and F is given in ml/cell/day.

2. The specific growth rate is calculated as follows: $K_c = \frac{1}{v}\frac{dv}{dt}$ and is given in divisions per cell per day.

3. The ^{14}C uptake is given as: $\frac{\text{counts/min}}{\mu\text{g Chl } a}$

4. See: TABLE 1 for media formulas.

5. Si: Twenty-eight mg per liter of $NaSio_3$-$9H_2O$ was added to the NH-15 medium prior to autoclaving (98.6 µg-At. Si/L).

6. The mean values were calculated for all population levels tested, excluding the highest and lowest values.

TABLE 17. SPECIFIC GROWTH RATE, CHL *a*/CELL AND ^{14}C UPTAKE OF *G. halli* EXPOSED TO VARIOUS TEMPERATURE-IRRADIATION COMBINATIONS

Irradiation mw/cm^2	Temperature °C	K_c[1]	Chl *a*/cell µg (X10^{-8})	^{14}C Uptake[2] (X10^4)
5	20	0.16	24.90	2.20
5	23	0.50	19.73	-
5	23	0.27	18.57	3.20
5	23	0.76	15.93	2.30
5	26	1.24	20.54	3.60
5	26	1.18	16.40	3.30
5	29	1.60	24.76	2.60
5	29	1.38	18.28	3.20
5	32	1.34	-	-
Mean[3]	-	0.95	19.71	3.03
10	20	0.42	23.30	2.80
10	23	0.47	19.36	3.40
10	23	0.50	16.57	4.40
10	23	0.46	17.49	2.70
10	23	0.93	15.29	3.70
10	26	1.18	19.39	3.90
10	26	1.37	16.64	3.40
10	29	1.42	21.50	3.30
10	29	1.50	18.32	3.40
10	32	1.54	-	-
10	32	1.45	21.70	3.10
10	35	0.0	-	-
Mean[3]	-	1.03	18.87	3.38
15	20	0.41	19.42	3.00
15	23	1.02	21.22	5.50
15	23	0.65	15.22	7.00
15	23	0.89	18.59	4.00
15	26	1.17	20.12	5.00
15	26	1.32	16.34	5.50
15	29	1.65	20.10	4.20
15	29	1.76	16.70	6.70
15	32	1.66	-	-
15	32	1.58	20.97	4.20
15	35	0.0	-	-
Mean[3]	-	1.24	18.89	5.06

Notes:

1. The specific growth rate is calculated as follows: $K_c = \frac{1}{v}\frac{dv+Vc}{dt}$ and is given in divisions per cell per day.

(continued)

TABLE 17 (continued)

2. The ^{14}C uptake is given as: $\frac{\text{counts/min}}{\mu\text{g Chl } a}$

3. The mean values were calculated for all temperatures in which cell division occurred, excluding the highest and lowest values.

different with certain light intensities. For example, at 20 and 32C the K_c (0.16 and 1.34) of the cultures receiving 5 mw/cm^2 irradiation were less than those that received 10 (0.42 and 1.54) and 15 mw/cm^2 (0.41 and 1.66) irradiation. The K_c values at 26C were not significantly different at the 95% confidence level at the three irradiation levels (TABLE 17).

The Chl *a*/cell was constant at each of the fifteen temperature-irradiation combinations tested. Results from analysis of variance indicated the Chl *a*/cell was similar (19.71, 18.87, 18.89) for cultures, irrespective of the light intensity-temperatures combination.

The ^{14}C uptake values did not vary significantly within the temperature range employed, but there was a significant difference of ^{14}C uptake values with increased light intensity.

Standard culture conditions for *G. halli* cultures for the remainder of this report are as follows:

1) Population level - 2.0 ± 0.5 x 10^4 cells/ml
2) Irradiation - 10 mw/cm^2
3) Temperature - 30C

The Chronic Effect of Mercury (Hg^{+2}) on the Growth of *I. galbana* and *G. halli*

The chronic effects of mercury on *I. galbana* were apparent only after the Hg concentration in the turbidostat culture exceeded 200 μg Hg/l. K_c values for *I. galbana* exposed to mercury, at concentrations less than 200 μg Hg/l, were similar to those of the control in which distilled water was continuously added in the place of the Hg solution. The K_c values decreased with a slope of -0.002 with mercury concentrations greater than 200 μg Hg/l. The Chl *a*/cell and ^{14}C uptake values did not change significantly throughout the range of mercury concentrations tested, in which cell division occurred (TABLE 18). A steady-state culture was not established with medium containing 602 μg Hg/l. After three days, cell division ceased and the cell population was not maintained. The Chl *a*/cell (2.17) after three days incubation in medium with 602 μg Hg/l was 47% less than the mean Chl *a*/cell value (4.09) for all other Hg concentrations tested. The ^{14}C uptake (1.34) after three days in medium with 602 μg Hg/l was 36% less than the mean ^{14}C uptake value (2.09) for all other Hg concentrations tested (TABLE 18).

The growth of *G. halli* was enhanced by the addition of less than 90 μg Hg/l (TABLE 19). The K_c was higher than that of the control (1.05) with additions of 11.9 μg Hg/l in which the maximum K_c was 1.99. The K_c was less in medium with higher Hg concentrations. Cell division ceased in medium with 121 μg Hg/l after three days. The maximum K_c of 1.99 with 11.9 μg Hg/l represented an 86% increase above that of the control value of 1.05.

The Chl *a*/cell and ^{14}C uptake of *G. halli* grown at 30C decreased significantly (95% confidence level) only after the concentration of mercury was more than 100 μg Hg/l (TABLE 19). The Chl *a*/cell (11.66) in medium with 107.3 μg Hg/l was 31% less than the mean Chl *a*/cell value (16.96) for all

TABLE 18. SPECIFIC GROWTH RATE, CHL *a*/CELL, AND ^{14}C UPTAKE OF *I. galbana* GROWN AT 23C WITH 10 MW/CM^2 IRRADIATION AND CONTINUOUS EXPOSURE TO VARIOUS CONCENTRATIONS OF $HgCl_2$

Hg^{+2} µg/L	K_c[1.]	Chl *a*/cell µg(X10^{-8})	^{14}C Uptake[2.] (X10^4)
2.8% H_2O	1.24	4.82	1.47
1.20	1.26	4.81	1.79
29.6	1.33	4.82	1.73
50.0	1.36	4.43	2.07
103.9	1.25	3.88	2.54
134.1	1.29	4.67	2.31
165.0	1.32	3.94	2.16
221.0	1.02	3.44	2.12
301.7	1.06	4.19	2.33
335.3	0.78	3.31	2.58
485.4	0.58	4.12	1.73
538.0	0.33	3.31	1.71
601.8	0.00	2.17	1.34
Mean	-	4.09	2.09

Notes:

1. The specific growth rate is calculated as follows: $K_c = \frac{1}{v}\frac{dv+Vc}{dt}$ and is given in divisions per cell per day.
2. The ^{14}C uptake is given as: $\frac{\text{counts/min}}{\mu\text{g Chl } a}$

TABLE 19. SPECIFIC GROWTH RATE, CHL *a*/CELLS, AND ^{14}C UPTAKE OF *G. halli* GROWN AT 30C WITH 10 MW/CM^2 IRRADIATION AND CONTINUOUS EXPOSURE TO VARIOUS CONCENTRATIONS OF Hg^{++}

Hg^{++} µg/L	K_c [1.]	Chl *a*/cell µg(X10^{-8})	^{14}C Uptake[2.] (X10^4)
3.4% H_2O	1.05	15.91	5.02
0.079	1.08	18.41	3.08
0.097	1.19	14.12	4.73
0.12	1.28	14.25	4.03
0.30	1.23	16.17	3.82
1.13	1.35	19.85	3.91
1.34	1.24	15.55	3.65
3.75	1.62	18.79	3.90
4.21	1.61	16.26	3.71
11.9	1.99	18.62	3.56
28.2	1.91	16.04	2.74
37.2	1.79	19.14	3.73
64.2	1.50	22.94	2.69
92.6	1.04	13.22	1.76
89.7	0.91	16.22	2.78
107.3	0.83	11.66	1.78
121.0	0.00	7.32[3.]	1.49[3.]
Mean[4.]	-	16.96	3.46

Notes:

1. The specific growth rate is calculated as follows: $K_c = \frac{1}{v}\frac{dv+Vc}{dt}$ and is given in divisions per cell per day.
2. The ^{14}C uptake is given as: $\frac{\text{counts/min}}{\mu g \text{ Chl } a}$
3. The values reported were from the 5th day of exposure at the indicated Hg concentration.
4. The mean values were determined for all Hg concentrations less than 100 µg/L, excluding the highest and lowest values.

test portions. The ^{14}C uptake in medium with 121 μg Hg/l of 1.49 was significantly less (95% confidence level) than the value of 1.78 in medium with 107.3 μg Hg/l, ^{14}C uptake value of 3.46.

Results of turbidostat experiments on effects of mercury on the growth of *G. halli* at 23C were similar to those observed at 30C (TABLE 19). The K_c rates were higher (1.27) in medium with Hg concentrations of 6.32 μg Hg/l but were less with 109 μg Hg/l. Cell division stopped in medium with 109 and 118 μg Hg/l. The maximum K_c value of 1.27 in medium with 6.32 μg Hg/l represented a 154% increase above the maximum control value of 0.50.

The Chl *a*/cell and ^{14}C uptake of *G. halli* grown at 23C with various concentrations of $HgCl_2$ changed significantly only after the mercury concentration exceeded 100 μg Hg/l. The Chl *a*/cell in medium with 109.1 and 118.0 μg Hg/l were 47 and 58% less than the mean value of 17.33 for values of test portions that contained less than 100 μg Hg/l (TABLE 20). The ^{14}C uptake value in medium with 118.0 μg Hg/l was 76% less than the mean ^{14}C uptake of 3.76 for cultures with 100 μg Hg/l or less.

An intensive study of the chronic effect of mercury on the growth of *G. halli* at 23C in medium with concentrations of 1.0 μg Hg/l or less, indicated an unusual pattern of growth response for the 13 concentrations of Hg tested (TABLE 20). The K_c (0.67) of *G. halli* grown in medium with the lowest mercury concentration tested (0.012 μg Hg/l) was 34% greater than the K_c of the control culture (0.50). The K_c increased linearly to a maximum of 0.79 with additions at 0.052 μg Hg/l. Subsequently, the K_c were less and continued to be less to a minimum of 0.51 at 0.462 and 0.652 μg Hg/l. The K_c increased to 0.72 in medium with 0.99 μg Hg/l (TABLE 20). The K_c was not less than the control (0.50) in medium with any of the 13 Hg concentrations tested.

The chronic effect of chromium on the growth of *I. galbana* at 23C with 10 mw/cm^2 irradiation was observed by determining changes in the K_c and ^{14}C uptake as the steady-state concentration of Cr was increased from 0.72 to 167.8 μg Cr/l. The K_c with Cr concentrations in medium of 2.21 μg Cr/l or less were not significantly (95% confidence level) different from the K_c (1.24) of the control culture. With Cr concentrations from 9.8 to 32.3 μg/l the growth of *I. galbana* was stimulated and the K_c was significantly (95% confidence level) higher than the K_c of the control culture (TABLE 21). The K_c maximum of 1.57 developed in medium with 11.2 μg Cr/l and the minimum K_c of 0.34 occurred in medium with the highest Cr concentration (167.8 μg/l) tested. The ^{14}C uptake of 1.47 (control culture) was less than that of 1.96, the lowest concentration of Cr (0.72 μg/l) tested. The K_c was less in the concentration of Cr (92.8 μg Cr/l). The Chl *a*/cell values did not change significantly (95% confidence level) from that of the control culture over the total range of Cr concentrations tested.

The growth of *G. halli* was stimulated at the lowest Cr concentration (0.04 μg Cr/l) tested and the K_c (1.16) was significantly (95% confidence level) higher than the K_c (1.01) of the control culture (TABLE 22). The K_c values were higher in medium with Cr concentrations of 0.2, 0.38, 3.8, 7.88, 12.0 and 12.8 μg Cr/l than in the control. The minimum K_c of 0.25 developed in

TABLE 20. SPECIFIC GROWTH RATE, CHL *a*/CELL, AND ^{14}C UPTAKE OF *G. halli* GROWN AT 23C WITH 10 MW/CM^2 IRRADIATION AND CONTINUOUS EXPOSURE TO VARIOUS CONCENTRATIONS OF Hg^{++}

Hg^{++} µg/L	K_c 1.	Chl *a*/cell µg$(X10^{-8})$	^{14}C Uptake 2. $(X10^4)$
2.7% H_2O	0.50	16.57	4.36
2.8	0.50	19.73	-
0.012	0.67	19.56	2.87
0.021	0.75	17.12	4.50
0.052	0.79	15.54	4.17
0.078	0.75	19.23	3.42
0.100	0.75	16.78	4.80
0.031	0.72	20.24	3.72
0.166	0.65	21.55	2.90
0.316	0.57	16.50	2.41
0.438	0.54	15.14	3.15
0.462	0.51	16.68	-
0.652	0.51	19.76	-
0.890	0.60	19.78	-
0.990	0.72	16.93	-
1.83	0.73	17.49	-
2.67	0.73	19.05	1.92
3.50	0.69	18.57	-
4.95	0.80	16.87	2.42
6.32	1.27	15.14	4.36
13.1	1.22	16.66	3.54
24.7	1.20	14.99	4.96
51.0	1.02	14.61	5.38
80.9	0.75	14.17	-
109.1	0.16	9.24	-
118.0	0.0	7.21	0.92
Mean 3.	-	17.33	3.76

Notes:

1. The specific growth rate is calculated as follows: $K_c = \frac{1}{v}\frac{dv+Vc}{dt}$ and is given in divisions per cell per day.
2. The ^{14}C uptake is given as: $\frac{\text{counts/min}}{\mu g \text{ Chl } a}$
3. The mean value was determined for all Hg concentrations less than 100 µg/L excluding the lowest and highest values.

TABLE 21. SPECIFIC GROWTH RATE, CHL *a*/CELL, AND ^{14}C UPTAKE OF *I. galbana* AT 23C WITH 10 MW/CM^2 IRRADIATION AND CONTINUOUS EXPOSURE TO VARIOUS CONCENTRATIONS OF $CrCl_3$

Cr^{+3} µg/L	K_c[1]	Chl *a*/cell µg(X10-8)	^{14}C Uptake[2] ($X10^4$)
2.8% H_2O	1.24	4.82	1.47
0.72	1.24	5.20	1.96
2.21	1.23	5.33	1.71
9.8	1.40	5.14	1.46
11.2	1.57	4.37	1.37
23.6	1.43	4.68	1.29
32.3	1.32	4.66	1.26
51.8	1.27	4.91	0.94
56.3	1.04	4.56	0.99
92.8	0.82	5.07	1.05
113.7	0.75	5.10	0.90
167.8	0.34	5.19	0.47
Mean[3]	-	4.95	-

Notes:

1. The specific growth rate is calculated as follows: $K_c = \frac{1}{v} \frac{dv+Vc}{dt}$ and is given in divisions per cell per day.
2. The ^{14}C uptake is given as: $\frac{\text{counts/min}}{\mu g \text{ Chl } a}$
3. The mean values were calculated for all chromium concentrations tested, excluding the highest and lowest values.

medium with the highest concentration (35.6 μg Cr/l) tested. The Chl *a*/cell of *G. halli* in media with Cr concentrations of 7.88 or less (19.57) was significantly higher than the Chl *a*/cell of the control culture (14.27) (95% confidence level). The minimum Chl *a*/cell was in medium with Cr concentrations of 35.06 μg Cr/L, the highest Cr concentration tested. The Chl *a*/cell maximum was 20.88 μg Chl *a* x 10^{-8} in medium that contained 0.20 μg Cr/L (TABLE 22). The ^{14}C uptake was not significantly changed (mean 2.80) by Cr concentrations of 25.3 μg Cr/L or less. With media concentrations of 28.4 and 35.6 μg/Chl *a* the ^{14}C uptake was significantly less (1.52 and 1.36, respectively) than the ^{14}C uptake (2.61) in medium with 25.3 μg Cr/l (TABLE 22).

TABLE 22. SPECIFIC GROWTH RATE, CHL *a*/CELL AND ^{14}UPTAKE OF *G. halli* AT 30C WITH 10 MW/CM^2 IRRADIATION AND CONTINUOUS EXPOSURE TO VARIOUS CONCENTRATIONS OF $CrCl_3$

Cr^{+3} μg/L	K_c [1.]	Chl *a*/cell μg(X10-8)	^{14}C Uptake[2.] (X10^4)
N/A	1.01	14.27	3.67
0.04	1.16	18.84	2.58
0.20	1.21	20.88	2.85
0.38	1.40	19.57	2.01
3.8	1.32	20.03	1.80
7.88	1.23	18.38	3.80
12.0	1.17	15.75	2.43
12.8	1.02	12.60	3.42
14.3	1.00	11.90	3.51
19.7	0.74	8.38	3.00
25.3	0.76	9.29	2.61
28.4	0.66	9.10	1.52
35.6	0.25	7.42	1.36
Mean[3.]	-	-	2.80

Notes:

1. The specific growth rate is calculated as follows: $K_c = \frac{1}{v}\frac{dv+Vc}{dt}$ and is given in divisions per cell per day.
2. The ^{14}C uptake is given as: $\frac{\text{counts/min}}{\mu g\ \text{Chl}\ a}$
3. The mean value was calculated for all concentrations of chromium up to and including 25.3 μg Cr/L, excluding the lowest and highest values.

SECTION 7

THE CHRONIC TOXICITY OF MERCURY, CHROMIUM AND NICKEL TO *G. halli* AND *I. galbana* (CHEMOSTAT)

The addition of a metal to a fixed volume of seawater may result in an instantaneous concentration of the metal in this medium that is near the amount added per unit volume. But, heavy metals react with components of seawater and form low solubility (approaching zero) products (Duursma, 1966) and the concentration of a specific metal that results from a given addition decreases with time. Thus, the effects of a specific concentration of a specific metal on a specific organism cannot be determined in this manner. The ionic concentration of metals can be increased by the use of chelators but this decreases the activity of the metal ions. A steady-state concentration of the metal that approaches the desired concentration can be established in a continuous-flow culture system. Continuous flow culture systems are principally of the chemostat (Novick and Sailard, 1950; Monad, 1950) or the turbidostat (e.g., Maddox and Jones, 1964; Freeberg, this report) types. But this steady-state condition can only be accomplished if the medium and metals solution, which is in a non-reacting solvent, are delivered in the proper amounts from separate reservoirs. The turbidostat differs from the chemostat in that the flow of the turbidostat decreases, increases or stops according to the growth of the population whereas the flow of a chemostat continues at the set rate independent of the growth of the population. The population controls the flow of the turbidostat and the flow of the chemostat is mechanically controlled at a specific rate. Studies were conducted on the toxicity of Hg and Cr to cultures of *Glenodinium halli* and *Isochrysis galbana* in a turbidostat and in a chemostat. Also, the toxicity of Ni to *G. halli* was studied in a chemostat. Results of turbidostat experiments were included in Section 6. of this report.

MATERIALS AND METHODS

The chemostat that was developed for metals toxicity tests consisted of two peristaltic pumps controlled by a Monroe Electronics Timer (Model 231 with Model 510-3 accessory) that synchronized the delivery of the test material and the medium from separate reservoirs through tubing to a 3-way, Y-type connector located above the culture vessel (Figure 4). We used Manostat, Harvard, Sigmamotor and Virtis peristaltic pumps. None of these were completely satisfactory, mainly because the rotor pinched or ruptured the tubing. The air was supplied from a Silent Giant Pump Model 120 (Aquarium Pump Supply, Inc.) through the third arm of the connector. The air supply was passed through a coil in a heated 6" x 6" chamber and filtered through a sterile fiberglass column and a Koby, Inc. Junior Air Purifier and Flow Equalizer and delivered through the third arm of the 3-way connector. The

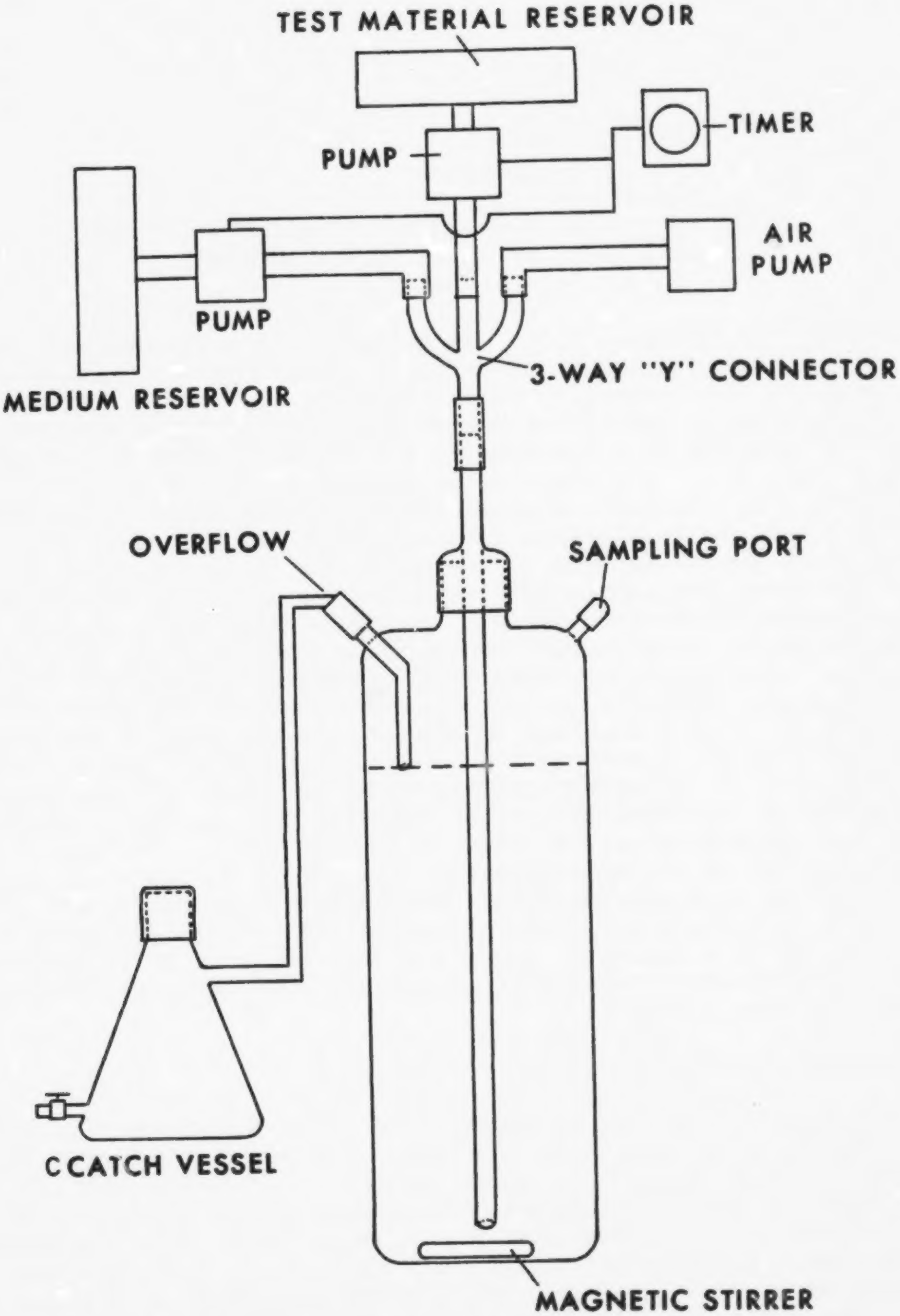
TEST MATERIAL RESERVOIR
TIMER
PUMP
AIR PUMP
PUMP
3-WAY "Y" CONNECTOR
MEDIUM RESERVOIR
OVERFLOW
SAMPLING PORT
CCATCH VESSEL
MAGNETIC STIRRER

Figure 4. Diagrammatic sketch of the basic chemostat design.

air pump could be operated continuously or activated at the same time that the pumps were activated by the timer for medium and test material delivery. Since our off-on cycle on the timer was 99 sec:5-12 sec., the air, medium and test material were supplied synchronously. This off-on cycle was more appropriate for the growth of cultures. It stirred the cultures adequately; facilitated the medium delivery; dissolved medium gas balance and overflow uniformity. The 3-way connector was mounted on a glass delivery tube that extended to the bottom of the culture vessel. Thus, the culture medium and test material came together immediately before delivery to the bottom of the culture vessel and was mixed into the culture volume by the turbulent air flow. This intermittent agitation was beneficial to the chemostat growth of dinoflagellates. The culture vessel was air-tight except through the overflow port at the surface of the culture volume. The overflow port could be accurately calibrated, with the dimension of our culture vessel, for any volume from 100 to 1200 ml by adjusting the overflow tube length. We employed a 1-liter volume. The aeration and a magnetic stirrer were used to maintain a homogeneous distribution of the culture, however, the stirrer was not required. The 1000 ft. c. light intensity for the culture was supplied by three 15-watt daylight fluorescent lights placed four inches from the culture vessel on each of three sides. The entire apparatus was housed in a walk-in constant temperature room. As many as six chemostats were in operation at one time with a single set of pumps and timers.

Initially the flow of the medium was 500 ml ± 50 ml per day and the flow of the test material (distilled water solution of $HgCl_2$, $CrCl_3$, or $NiCl_2$) was 5 ml ± 0.5 ml per day. Rates were established by the timer, the pump rate and the size of the tubing. Subsequently, the flow rate of both materials were doubled so that there was one complete volume exchange per day.

Sampling for population counts and Chlorophyll *a* measurements were made at the same time daily by catching the overflow during a one-half to one hour period. The overflow vessel was an integral part of the system and was maintained aseptically. Cell counts were made with a Model B Coulter Counter and Chlorophyll *a* was measured with a modification of the fluorometric method of Strickland and Parsons (1968) as modified (p. 43, Section 6 of this report). The test solutions of Hg, Ni or Cr were prepared according to the concentration desired in the culture vessel so that 10 ml of this solution mixed with one liter of culture medium resulted in the desired concentration. This metal test solution, prepared with distilled water was contained in a graduated cylinder. The cylinder was closed with a two-hole silicone stopper. The volume delivered each day from this reservoir was subtracted from the total overflow volume from the culture to determine the amount of medium and test material solution delivered.

The medium was prepared in 8-liter amounts in 2.5 gallon Pyrex carboys closed with a two-holed, shirted stopper (Baltimore Biological Laboratory). Both thick-wall silicone and viton tubing was used for all connections between reservoirs, culture vessels, air filters and catch-vessels. The medium or test solutions as well as components of the system that were in contact with them were autoclaved at either 15 psi for 15 minutes or for 30 minutes for the 8-liters of medium. All connections were made prior to autoclaving, if possible. Otherwise all connecting terminals were wrapped with gauze and masking tape and each connection made aseptically, in the constant temperature room after autoclaving. Glassware cleaning procedures were described on p. 43, Section 6.

All experiments with *G. halli* were conducted in medium with 28 ppt salinity at 28C and those with *I. galbana* were conducted at 28 ppt salinity and 12C as controls. Sterility tests were made with Spencers (1952) seawater peptone and peptone seawater agar every third day of operation, at the time of a change in the concentration of the metal tested, at the finish of a run or at other times that sterility was questionable.

RESULTS

Results of some of the first experiments conducted with *G. halli* indicated that Hg, Cr and Ni were much more toxic in a chemostat than in the static acute toxicity assays (Figures 5 and 6). Concentrations as low as 50 mμg Hg/L, 0.1 μg Cr/L and 0.2 μg Ni/L reduced population levels and the Chlorophyll *a* content by an order of magnitude or more within 16 days or less. Although these tests were repeated either three or four times, these metal concentrations did not again elicit these high toxic effects. Also, lower concentrations had no effects (Figures 7 and 8). Indeed, the concentration of the metals that were toxic subsequently were similar to or less than an order of magnitude less than the LMTL values of the acute toxicity tests with both *G. halli* and *I. galbana* (TABLE 2).

Other than these initial experiments conducted to determine the toxicity of Hg to *G. halli* (Run A and B, TABLE 23), population levels of *G. halli*, as indicated by cell counts and Chlorophyll *a*, were maintained in media with concentrations from 0.01 to 10.0 μg Hg/L (Run 1 and 2, TABLE 23). Collectively, these results indicate that the minimum concentration that was toxic to *G. halli* in this system was from 20 to 100 μg Hg/L. The mean population levels for the periods that the system contained 0.08 to 10 μg Hg/L were significantly higher than during the periods that the medium contained 0.02 μg Hg/L or less (Run 1, TABLE 23). These results may indicate that these concentrations of Hg were stimulatory, but this effect did not occur with 5 and 10 μg Hg/L in another test (Run 2, TABLE 23).

The toxic levels of Cr to *G. halli* were between 10 and 100 μg Cr/L. Exposures of 5 to 12 days to media that contained from 0.01 to 100 μg Cr/L did not reduce the cell number or Chlorophyll *a* content significantly (Run 1 and 2, TABLE 24) and noticeable population changes occurred only after the Cr level was raised to 1000 μg Cr/L. On the other hand, in subsequent tests, the toxic level was 100 μg Cr/L or less (Runs 3 and 4, TABLE 24). Less than 100 μg Cr/L reduced the number of organisms in another series (Run A, TABLE 24), but the population levels and Chlorophyll *a* content declined throughout the 35-days test, possibly caused by something other than the Cr concentration. The toxicity level of Ni to *G. halli* was approximately 50 μg Ni/L. Concentration of 0.05 to 40 μg Ni/L did not cause a significant change in the population numbers or Chlorophyll *a* concentrations. On the other hand, concentrations of 50 μg Ni/L caused a significant decrease in both measurements (Run 1, 2 and A, TABLE 25) and a concentration of 100 μg Ni/L caused a

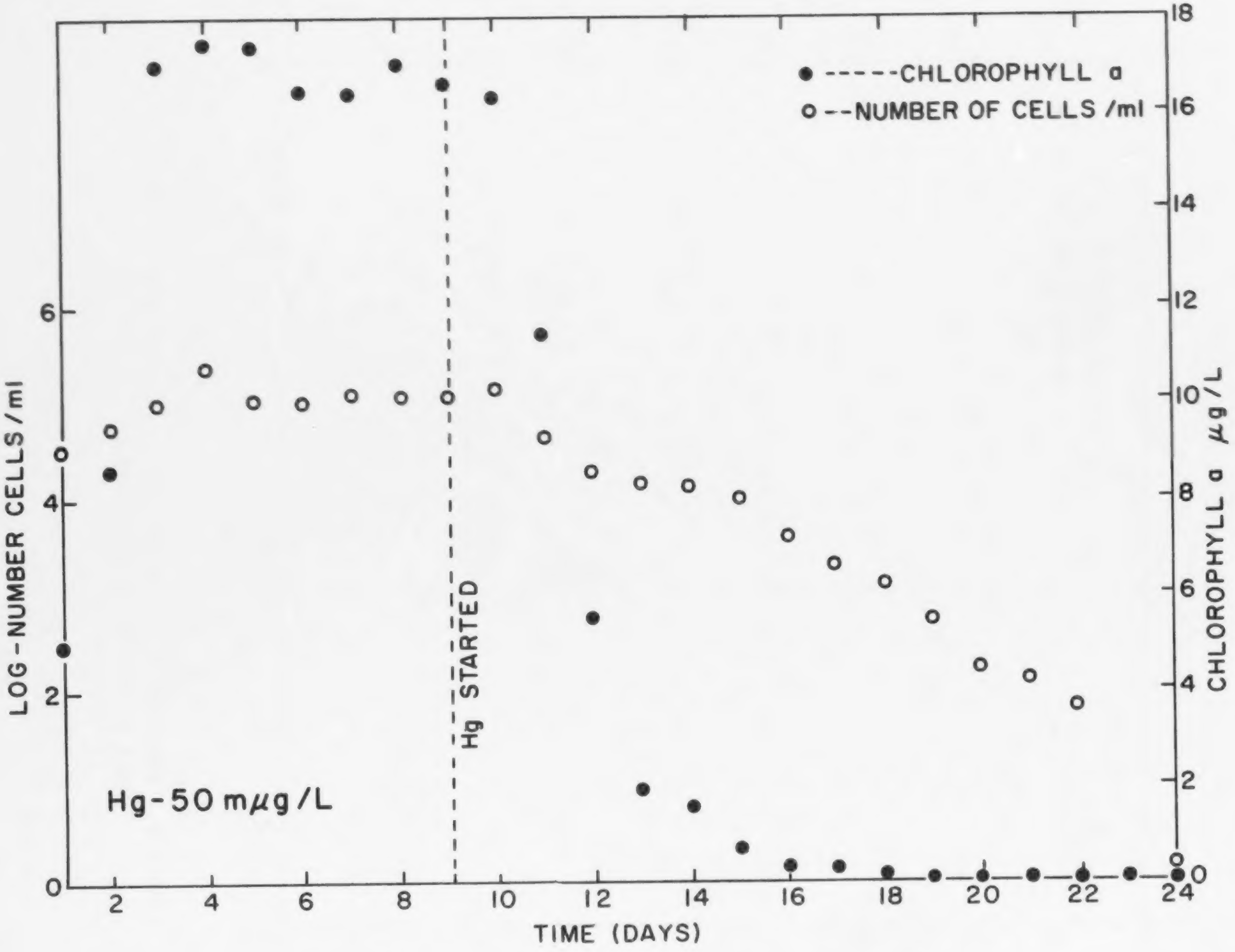

Figure 5. Response of *G. halli* culture to Hg concentration of 50 mμg/L (one experiment).

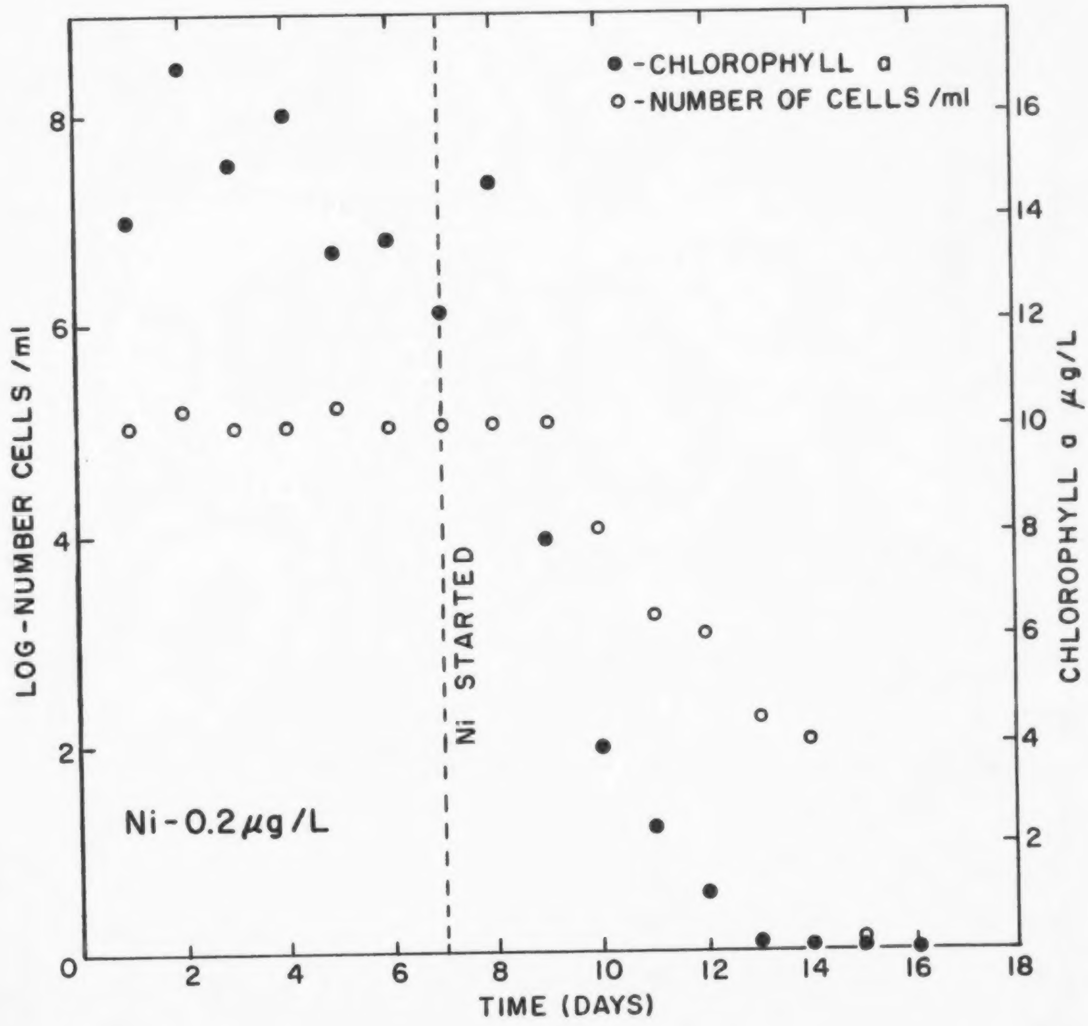

Figure 6. Response of *G. halli* culture to Ni concentration of 0.2 μg/L (one experiment).

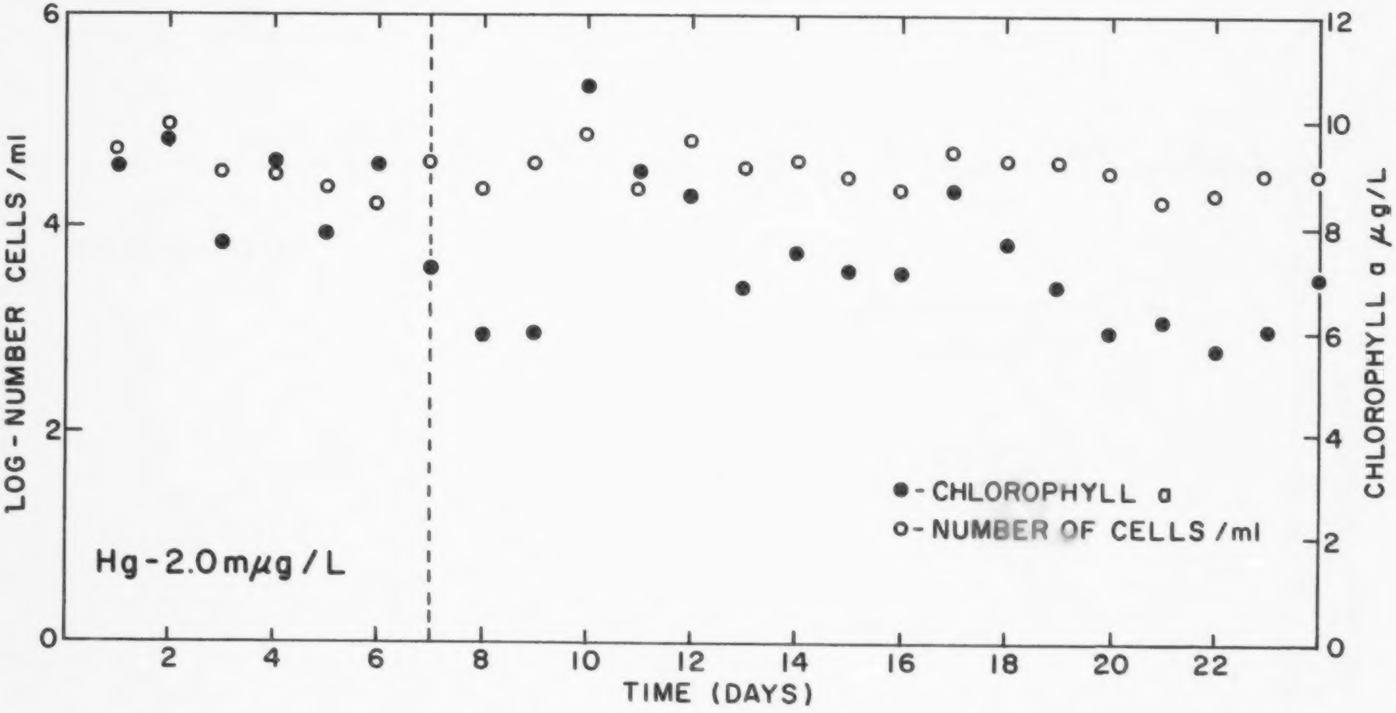

Figure 7. Response of *G. halli* culture to Hg concentration of 2 mµg/L (one experiment).

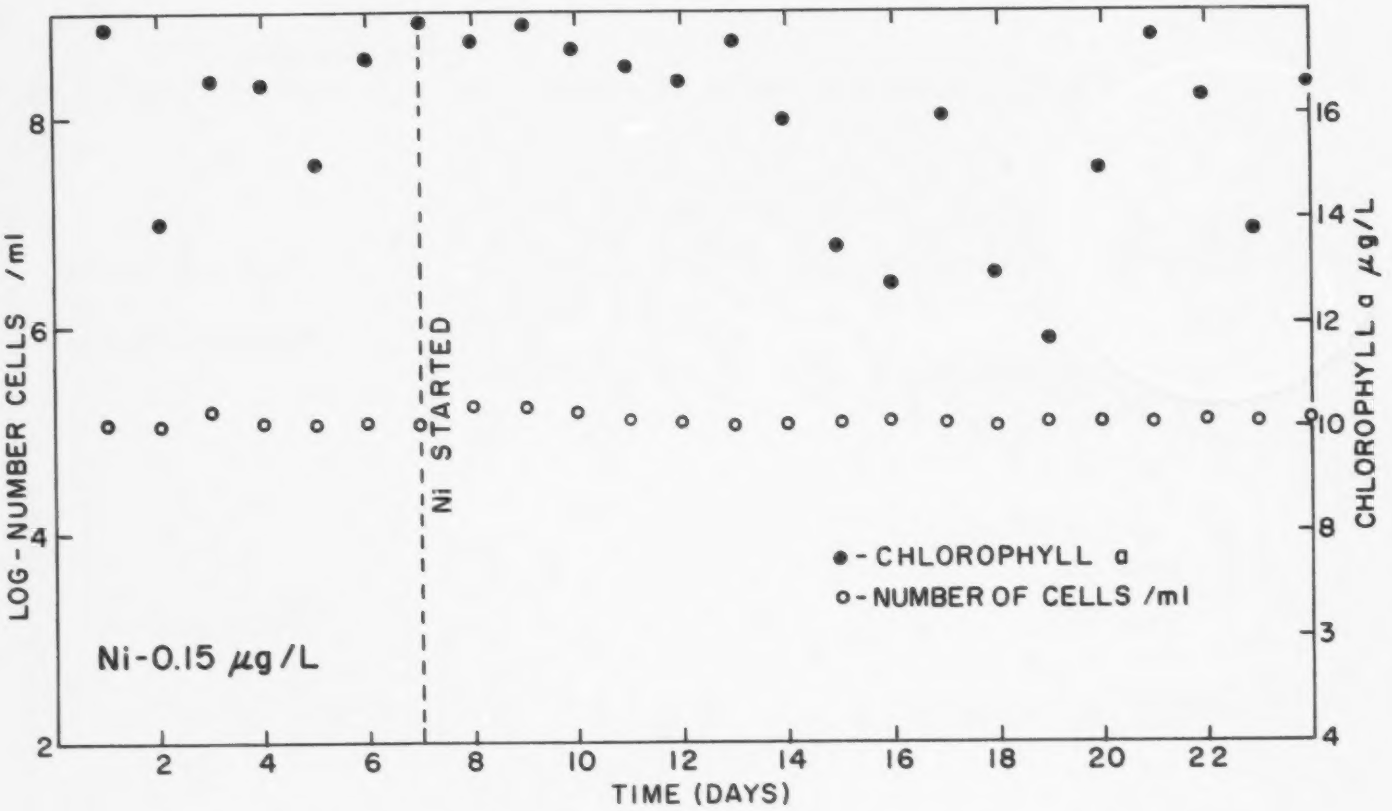

Figure 8. Response of *G. halli* culture to Ni concentration of 0.15 μg/L (one experiment).

more rapid decline in the number of organisms and Chlorophyll *a*.

Chronic effects of Hg to *I. galbana* indicated that the toxicity level was 100 µg Hg/L, or possibly less. The first test series with *I. galbana* and Hg with concentrations from 0.02 to 800 µg Hg/L indicated that the toxicity level was between 80 and 800 µg Hg/L (Run 1, TABLE 26). Although the mean population number and Chlorophyll *a* of the culture that received 80 µg Hg/L was less than the one that received the next lower amount (8 µg Hg/L), the difference was not significant. In the second test series, a concentration of 100 µg Hg/L caused a significant population decrease within 12 days and the population decreased more rapidly in medium with 200 µg Hg/L (Run 2, TABLE 26).

The minimum concentration of Cr that produced a decline of *I. galbana* populations in a chemostat was between 5 and 100 µg Cr/L. Populations were reduced by concentrations of 100 µg Cr/L (Runs 2 and 3, TABLE 27). The mean population numbers and Chlorophyll *a* levels were significantly less in media with 5 to 40 µg Cr/L than during periods that the media contained 0.5 µg Cr/L or less. These differences may be meaningless as the population continued to grow at a reduced rate, but there was a general downward trend of both cell numbers and Chlorophyll *a* of populations in media that contained progressively higher concentrations of Cr from 5 to 40 µg Cr/L. Both experiments indicate that 100 µg Cr/L was toxic to *I. galbana* (Runs 2 and 3, TABLE 27).

Nickel was toxic to chemostat cultures of *I. galbana* at concentrations between 40 and 100 µg Ni/L. Concentrations of 40 µg Ni/L or less had no effect on the growth of *I. galbana* as indicated by the cell counts or Chlorophyll *a* concentrations but the populations decreased in media that received 80 µg Ni/L or more (Run 2 and 3, TABLE 28). Another test (Run 1, TABLE 28) indicated that 0.2 to 0.4 µg Ni/L may have been toxic to *I. galbana* but subsequent experiments did not show that these concentrations reduced the populations of the system.

At the termination of each run of a chemostat experiment, cultures of *G. halli* and *I. galbana* usually grew if allowed to remain in the culture vessel in a static system. At the end of the incubation periods, the cultures were examined to determine whether or not the population level had increased. After most initial inhibitory effects were observed, the air, medium and metal solution flows were stopped and the culture remained in a static incubation state for 8 to 15 days. Although the cells were enumerated and the relative Chlorophyll *a* was determined, no attempts were made to determine growth rates during this period. The results of these examinations show that *I. galbana* populations increased in all systems after the flow was stopped and that *G. halli* often, but not always, increased during this period. For the most part, *G. halli* increased in all cases except those in which the media contained metal concentrations that were much higher than the minimum toxicity level (e.g. 500 µg Hg/L, TABLE 23 and 1000 µg Cr/L, TABLE 24). On the other hand, *G. halli* populations died in media with relatively low metal concentrations, e.g., .05 µg Hg and 2 µg Hg (Runs A and B, TABLE 23, 30 µg Cr/L (Run A, TABLE 24) and 50 µg Ni/L (Run A, TABLE 25).

TABLE 23. CHRONIC TOXICITY OF MERCURY TO *G. halli* IN A CHEMOSTAT

						Run 1						
Hg added µg/L	0	.01	02	04	08	.1	1.0	2.0	4.0	10	100	500
Days of operation	7	10	10	10	11	7	8	6	4	5	6	4
1st day	6.15	8.26	6.94	7.85	10.24	12.70	12.06	12.20	13.45	13.96	9.46	0.48
Last day	6.68	8.31	8.21	10.32	12.45	11.15	12.99	12.35	13.52	12.52	4.89	0.01
$\overline{X}$ Ch *a*	7.23	7.46	5.94	8.43	12.32	11.83	11.88	12.51	13.32	13.45	7.23	0.25
σ Ch *a*	0.73	0.95	1.68	1.00	1.31	1.10	0.74	0.73	0.28	1.05	2.22	0.33
Var.Ch a	0.44	0.80	2.70	0.89	1.15	1.04	0.48	0.46	0.06	0.88	3.70	0.06
No. x 10^7 1st day	2.9	3.9	2.4	3.7	6.1	7.1	6.1	9.0	7.3	9.7	6.8	0.50
No. x 10^7 Last day	3.8	3.0	3.4	5.0	7.1	6.0	8.9	6.5	9.3	9.9	4.3	0.30
$\overline{X}$ No.cells	3.6	2.79	2.70	2.92	7.31	6.60	7.61	7.57	8.12	9.36	6.10	0.40
σ No.cells	0.56	0.75	0.70	0.56	0.70	0.77	0.84	0.84	0.84	0.42	1.22	0.14
Var. No. cells	0.27	0.52	0.44	0.28	0.44	0.51	0.62	0.58	0.53	0.14	1.12	0.01

(continued)

TABLE 23. (continued)

	Run 2				Run A		Run B		
Hg added µg/L	0	5	10	20	0	.05	0	1.0	2.0
Days of operation	6	11	10	4	8	16	9	10	15
1st day	13.60	11.13	9.57	4.90	26.43	24.41	3.2	9.8	14.9
Last day	11.35	10.02	7.75	0.03	24.02	0.02	7.8	15.2	0.1
$\bar{X}$ Ch *a*	12.65	10.06	8.77	2.51	23.42	6.31	5.65	14.67	6.66
σ Ch *a*	0.94	1.53	1.10	2.47	3.10	11.71	1.45	2.57	6.18
Var.Ch *a*	0.73	2.14	1.08	4.59	8.17	128.5	1.85	5.94	35.69
No. x 10^7 1st day	6.9	6.9	5.1	3.3	20.0	18	1.7	5.2	13.0
No. x 10^7 Last day	6.3	4.4	4.2	0.38	18.0	0.13	4.2	14.0	0.8
$\bar{X}$ No. cells	6.52	4.72	4.85	2.27	16.8	5.19	2.86	9.93	6.26
σ No. cells	0.37	1.26	0.52	1.35	0.28	5.60	0.87	2.90	4.37
Var. No. cells	0.11	1.45	0.24	1.38	0.07	28.93	0.70	7.59	17.85

TABLE 24. CHRONIC TOXICITY OF CHROMIUM TO *G. halli* IN A CHEMOSTAT

	Run 1						Run 2					
Cr added µg/L	0	.01	02	04	08	.2	0.1	1.0	2.0	10	100	1000
Days of operation	9	10	11	9	12	5	11	5	4	5	5	3
1st day	9.56	15.02	12.40	15.49	14.40	11.84	17.12	14.55	12.79	15.29	11.61	4.21
Last day	13.31	13.51	15.92	13.30	11.79	13.82	14.56	12.35	14.05	13.43	13.43	0.53
$\overline{X}$ Ch *a*	11.38	11.89	12.66	13.91	13.76	13.98	14.67	13.28	13.55	14.05	12.75	2.07
σ Ch *a*	2.03	1.91	2.73	2.55	1.80	1.59	1.16	0.92	1.18	0.79	0.99	1.91
Var.Ch *a*	3.67	3.25	6.74	5.79	2.91	2.02	1.22	0.72	1.04	0.53	0.65	2.44
No. x 10^7 1st day	5.2	9.0	5.1	6.9	7.8	9.1	8.6	6.5	5.8	7.2	4.6	4.8
No. x 10^7 Last day	5.1	4.9	7.1	9.1	8.4	8.6	7.9	5.8	5.6	6.0	5.0	3.5
$\overline{X}$ No.cells	5.11	4.8	5.90	7.74	9.26	9.04	8.25	6.28	5.98	6.18	4.87	4.0
σ No. cells	0.65	1.70	1.55	0.80	0.76	0.47	0.53	0.38	0.33	0.72	0.23	0.7
Var. No. cells	0.38	2.59	2.19	0.58	0.53	0.18	0.25	0.12	0.08	0.04	0.04	0.32

(continued)

TABLE 24. (continued)

	Run 3		Run 4			Run A			
Cr added µg/L	0	100	0	10	20	0	1	15	30
Days of operation	14	8	9	8	7	8	11	14	2
1st day	24.93	16.03	21.71	20.19	15.48	16.03	13.13	2.67	0.14
Last day	15.14	0.24	18.13	18.00	0.45	11.80	4.40	0.20	0.16
$\overline{X}$ Ch *a*	21.67	7.47	18.73	19.15	5.47	14.38	11.1	1.36	0.15
σ Ch *a*	2.35	5.96	2.21	0.8	6.03	2.96	2.90	0.82	0.09
Var.Ch *a*	3.41	28.61	4.36	0.56	31.2	7.3	7.7	0.61	0.10
No. x 10^7 1st day	11.0	8.1	11.0	10.0	11.0	14.0	8.1	2.7	0.61
No. x 10^7 Last day	8.7	1.3	11.0	13.0	3.5	8.1	2.7	0.80	0.69
$\overline{X}$ No.cells	12.0	5.8	9.97	12.5	6.14	10.5	6.92	1.68	0.65
σ No.cells	1.42	2.48	1.17	1.6	3.22	2.16	1.80	1.10	0.06
Var. No. cells	1.31	7.80	1.22	2.25	8.90	3.89	2.87	1.16	0.00

TABLE 25. CHRONIC TOXICITY OF NICKEL TO *G. halli* IN A CHEMOSTAT

Run 1

Ni added µg/L	0	0.05	.1	.2	.4	1	5	20	40	50	100
Days of operation	7	11	12	9	12	7	10	6	6	5	3
1st day	13.67	10.86	12.32	12.18	11.38	13.88	13.92	20.69	12.35	11.21	0.35
Last day	11.38	11.01	12.63	12.67	12.52	12.06	20.51	13.67	15.74	2.01	0.18
$\overline{X}$ Ch *a*	12.58	9.61	9.84	12.18	13.9	12.88	17.92	15.60	14.20	5.26	0.21
σ Ch *a*	1.03	1.00	2.14	0.79	1.41	0.71	2.36	3.43	1.45	3.40	0.12
Var.Ch *a*	0.91	0.89	4.19	0.55	1.82	0.43	5.00	9.82	1.57	12.79	0.01
No. x 10^7 1st day	6.4	5.6	5.5	5.4	7.4	9.7	8.7	11.0	7.5	5.9	1.5
No. x 10^7 Last day	6.9	5.0	5.7	8.1	8.2	8.4	12.0	7.3	9.2	2.6	1.5
$\overline{X}$ No.cells	6.8	4.65	3.92	5.94	8.84	8.89	10.47	8.65	8.30	3.82	1.4
σ No.cells	0.50	0.61	1.16	1.05	1.10	0.66	1.22	1.30	0.79	1.38	0.17
Var. No. cells	0.21	0.33	1.23	0.99	1.11	0.37	1.35	1.41	0.47	1.53	0.02

(continued)

TABLE 25. (continued)

	Run 2				Run A		
Ni added µg/L	0	10	20	50	0	10	50
Days of operation	5	13	12	15	8	12	8
1st day	27.63	19.58	16.03	16.44	12.69	9.57	2.36
Last day	20.58	13.52	16.56	6.94	9.79	3.07	0.06
$\overline{X}$ Ch *a*	23.11	16.04	13.42	11.3	11.52	12.48	0.37
σ Ch *a*	3.07	1.99	3.22	3.61	2.48	7.84	0.80
Var.Ch *a*	7.52	3.65	9.53	12.51	5.15	56.39	0.57
No. x 10^7 1st day	16.0	12.0	8.6	9.8	6.1	5.3	2.3
No. x 10^7 Last day	13.0	6.7	9.5	4.7	6.2	4.0	0.6
$\overline{X}$ No.cells	14.0	8.77	6.97	5.95	6.4	8.35	1.02
σ No.cells	1.40	1.90	2.03	1.67	1.40	4.14	0.77
Var. No. cells	1.60	3.32	3.78	2.59	1.63	15.74	0.51

TABLE 26. CHRONIC TOXICITY OF MERCURY TO *I. galbana* IN A CHEMOSTAT

	Run 1												Run 2		
Hg added µg/L	0	.02	.04	.08	.2	.5	1	2	4	8	80	800	0	100	200
Days of operation	6	8	9	10	21	7	9	5	5	4	6	9	6	12	9
1st day	6.03	16.17	18.32	15.21	16.52	6.37	7.17	10.24	13.67	11.94	9.42	8.65	24.69	21.37	6.12
Last day	9.22	19.52	19.82	17.32	4.78	7.45	12.61	11.47	11.47	10.32	7.81	0.77	24.70	11.76	0.86
$\bar{X}$ Ch *a*	8.60	21.43	20.24	17.50	6.64	6.14	11.51	12.03	13.48	10.90	8.47	3.12	23.18	16.13	3.20
σ Ch *a*	1.91	4.86	1.90	1.17	3.40	1.37	2.59	1.46	0.39	0.80	0.55	2.62	1.20	4.74	1.91
Var.Ch *a*	3.05	20.70	3.20	1.12	10.97	1.61	5.95	1.59	0.11	0.49	0.25	6.00	1.20	20.6	3.26
No. x 10^7 1st day	1.5	4.1	2.7	3.0	2.4	1.6	1.2	1.5	2.1	1.6	1.4	1.3	10.0	3.1	1.2
No. x 10^7 Last day	2.7	3.9	5.4	2.8	0.99	2.0	2.0	1.8	1.7	1.7	1.4	0.27	5.0	1.6	0.3
$\bar{X}$ No.cells	2.7	4.54	3.66	3.32	1.36	1.44	1.88	1.83	1.86	1.63	1.33	0.61	6.37	3.17	0.75
σ No.cells	1.4	0.83	1.03	0.46	0.64	0.49	0.47	0.25	0.17	0.05	0.08	0.33	1.95	1.19	0.36
Var. No. cells	1.64	0.62	0.95	0.19	0.40	0.21	0.19	0.05	0.02	0.00	0.00	0.10	3.17	1.30	0.11

TABLE 27. CHRONIC TOXICITY OF CHROMIUM TO *I. galbana* IN A CHEMOSTAT

Cr added µg/L	Run 1			Run 2										
	0	005	01	0	02	04	08	.5	5	10	20	40	100	1 mg
Days of operation	6	13	10	10	11	9	10	15	11	7	8	8	7	5
1st day	14.66	17.07	13.81	17.07	16.76	16.50	21.86	18.38	17.42	6.60	5.01	2.69	6.38	2.65
Last day	17.64	14.32	12.52	19.92	13.81	20.21	21.69	17.72	7.62	4.12	1.37	5.89	1.63	1.07
$\bar{X}$ Ch *a*	16.26	22.03	14.21	18.48	18.04	22.23	21.34	20.05	8.27	4.28	3.73	3.54	3.96	1.55
σ Ch *a*	2.10	6.36	1.10	1.81	2.65	3.23	1.51	2.50	4.52	1.14	1.31	1.42	2.26	0.64
Var. Ch *a*	3.66	37.3	1.10	2.94	6.30	9.13	2.08	5.35	18.64	1.11	1.50	1.76	4.37	0.32
No. x 10^7 1st day	4.6	4.8	2.3	7.0	4.0	3.8	3.6	2.1	2.3	1.3	1.0	0.90	1.0	0.42
No. x 10^7 Last day	4.0	2.5	2.5	3.2	2.2	3.9	2.7	2.2	1.2	1.1	0.41	0.91	0.32	0.42
$\bar{X}$ No.cells	3.35	4.3	2.5	4.85	3.82	4.75	2.99	2.51	1.32	0.91	0.74	0.72	0.68	0.42
σ No.cells	1.15	1.60	0.40	1.54	0.98	0.93	0.48	0.37	0.48	0.23	0.22	0.15	0.38	0.04
Var. No. cells	1.10	2.30	0.18	2.13	0.88	0.75	0.21	0.13	0.21	0.05	0.04	0.02	0.13	0.00

(continued)

TABLE 27 (continued)

	Run 3	
Cr added μg/L	0	100
Days of operation	6	22
1st day	20.26	26.47
Last day	26.25	0.31
$\overline{X}$ Ch *a*	24.29	7.39
σ Ch *a*	2.86	10.17
Var. Ch *a*	6.80	98.75
No. x 10^7 1st day	5.7	6.5
No. x 10^7 Last day	9.1	0.12
$\overline{X}$ No.cells	6.63	1.73
σ No.cells	1.46	2.17
Var. No. cells	1.79	4.50

TABLE 28. CHRONIC TOXICITY OF NICKEL TO *I. galbana* IN A CHEMOSTAT

	Run 1			Run 2										
Ni added μg/L	0	.2	.4	0	.1	.2	.5	1	5	10	20	40	80	160
Days of operation	9	15	11	11	11	9	12	11	8	8	8	8	9	9
1st day	42.04	25.93	12.01	9.70	9.26	6.37	13.45	13.18	5.23	6.05	12.41	10.01	10.26	2.06
Last day	35.85	10.21	5.23	10.24	7.74	14.62	14.07	11.15	5.69	11.23	9.9	12.35	2.61	2.73
$\bar{X}$ Ch *a*	38.43	17.48	8.77	8.41	8.55	11.82	13.91	12.27	5.24	8.30	9.48	11.10	4.91	2.40
σ Ch *a*	4.50	5.30	2.32	1.99	1.79	3.78	1.70	0.98	.85	2.16	1.82	1.07	3.98	.28
Var.Ch *a*	18.88	23.88	4.90	3.59	2.88	12.74	2.66	0.87	.64	4.08	2.9	1.00	14.08	.06
No. x 10^7 1st day	6.0	5.2	2.3	2.6	2.1	1.6	3.2	3.0	0.88	1.2	2.8	2.0	1.8	.83
No. x 10^7 Last day	8.1	2.1	2.1	2.5	1.7	4.3	3.2	2.1	1.4	2.7	2.1	2.2	.74	.74
$\bar{X}$ No.cells	6.49	3.48	2.06	2.49	2.74	3.63	3.33	3.04	1.37	1.86	2.0	2.18	1.12	.71
σ No.cells	1.18	1.15	.38	.65	.78	1.37	.64	.58	.38	.61	.46	.36	.57	.1
Var. No. cells	1.14	1.23	.13	.38	.56	1.68	.38	.31	.12	.33	.18	.11	.29	.01

(continued)

TABLE 28 (continued)

	Run 3	
Ni added µg/L	0	100
Days of operation	6	20
1st day	13.58	12.69
Last day	13.13	0.33
$\bar{X}$ Ch *a*	14.16	5.57
σ Ch *a*	.93	5.65
Var.Ch *a*	.72	30.37
No. x 10^7 1st day	7.7	2.9
No. x 10^7 Last day	3.0	.43
$\bar{X}$ No.cells	5.6	1.62
σ No.cells	2.07	1.26
Var. No. cells	3.59	1.51

REFERENCES

Albert, A. 1950. Quantitative Studies of the Avidity of Naturally Occurring Substances for Trace Metals. I. Amino Acid Having Only Two Ionizing Groups. Biochem. J. 47:531-538.

Albrecht, W. A. 1941. Soil Organic Matter and Ion Availability for Plants. Soil Science 51:487-494.

Aldrich, D. V., and W. B. Wilson. 1960. The Effect of Salinity on Growth of *Gymnodinium breve* Davis. Biol. Bull. 119:57-64.

Alexander, J. E., and E. F. Corcoran. 1967. The Distribution of Copper in Tropical Sea Water. Limnol. Oceanogr. 12:236-242.

American Society of Testing and Materials. 1965. Manual on Industrial Water and Industrial Waste Water. ASTM Special Tech. Publ. No. 148-H.

Atkins, W. R. G. 1932. The Copper Content of Sea Water. J. Mar. Biol. Assn. U.K. 18:193-197.

Barber, R. T., and J. H. Tyther. 1969. Organic Chelators: Factors Affecting Primary Production in the Cromwell Current Upwelling. J. Exp. Mar. Biol. Ecol. 3:191-199.

Barker, H. A. 1935. The Culture and Physiology of Marine Dinoflagellates. Archiv für Mikrobiologie 6:157-181.

Barnes, H. A., and F. A. Stanbury. 1948. The Toxic Action of Copper and Mercury Salts Both Separately and When Mixed on the Harpacticoid Copepod, *Nitocra spinipes* (Boeck). J. Exp. Biol. 25:270-275.

Barnes, H. A., and S. M. Marshall. 1951. On the Variability of Replicate Plankton Samples and Some Application of "Contagious" Series to the Statistical Distribution of Catches Over Restricted Periods. J. Mar. Biol. Assn. U.K. 30:233-263.

Ben-Bassat, D., G. Shelef, N. Gruner, and H. I. Shuval. 1972. Growth of Chlamydamonas in a Medium Containing Mercury. Nature, London 240: 43-44.

Bengough, G. D., and R. May. 1924. Seventh Report to the Corrosion Research Committee. J. Inst. Metals 32:81.

Brown, I. C., and M. Drosdoff. 1940. Chemical and Physical Properties of Soils and of Their Colloids Developed from Granitic Materials in the Mojave Desert. J. Agri. Res. 61:335-352.

Caperon, J. 1968. Population Growth Response of *Isochrysis galbana* to Nitrate Variation at Limiting Concentrations. Ecology 49:866-871.

Caperon, J., and J. Meyer. 1972. Nitrogen-limited Growth of Marine Phytoplankton. II. Uptake Kinetics and Their Role in Nutrient Limited Growth of Phytoplankton. Deep-Sea Res. 19:619-632.

Carpenter, E. J. 1970. Phosphorus Requirements of Two Plankton Diatoms in Steady State Cultures. J. Phycol. 6:28-30.

Carpenter, K. E. 1927. The Lethal Action of Soluble Metallic Salts on Fishes. J. Exp. Biol. 4:378-390.

Chow, T. J., and T. G. Thompson. 1952. The Determination and Distribution of Copper in Sea Water. Part I, The Spectrographic Determination of Copper in Sea Water. J. Mar. Res. 11:124-138.

Chow, T. J., and T. G. Thompson. 1954. The Seasonal Variation in the Concentration of Copper in the Surface Waters of San Juan Channel Washington. J. Mar. Res. 13:233-244.

Clarke, G. L. 1947. Poisoning and Recovery in Barnacles and Mussels. Biol. Bull. 92:73-91.

Collier, A. 1953. Titanium and Zirconium in Blooms of *Gymnodinium brevis* Davis. Science 118:329.

Collier, A., S. M. Ray, A. W. Magnitzky and J. O. Bell. 1953. Effects of Dissolved Organic Substances on Oysters. U.S. Fish Wildl. Serv., Fish. Bull. 84, 54:167-185.

Cook, S. F. 1925. A Latent Period in the Action of Copper on Respiration. J. Gen. Physiol. 9:631-651.

Cooper, L. N. H. 1935. Iron in the Sea and Marine Plankton. Proc. Royal Soc., London, Ser. B, 118:419-438.

Corcoran, E. F., and J. E. Alexander. 1964. The Distribution of Certain Trace Elements in Tropical Sea Water and Their Biological Significance. Bull. Mar. Sci. Gulf & Caribb. 14:594-602.

Davies, A. G. 1974. The Growth Kinetics of *Isochrysis galbana* in Cultures Containing Sublethal Concentrations of Mercuric Chloride. J. Mar. Biol. Assn. U.K. 54:157-170.

Davies, A. G. 1976. An Assessment of the Basis of Mercury Tolerance in *Dunaliella tertiolocta*. J. Mar. Biol. Assn. U.K. 56:39-57.

Davis, H. C., and R. R. Guillard. 1958. Relative Value of Ten Genera of Micro-Organisms as Food for Oyster and Clam Larvae. U.S. Fish Wildl. Serv., Fish. Bull. 58:293-394.

Davy, Sir Humphrey. 1825. Additional Experiments and Observations on the Application of Electrical Combinations to the Preservation of the Copper Sheating of Ships, and to Other Purposes. Philos. Trans. Royal Soc., London, 1825, pp. 242-246. In: Woods Hole Oceanographic Institution, 1952, Marine Fouling and Its Prevention, U.S. Naval Inst., Annapolis, Md.

de Kock, P. C. 1955. Influence of Humic Acid on Plant Growth. Science 121:473-474.

Dittmar, W. 1884. Cited from: Sverdrup, H. U., M. W. Johnson, and R. H. Fleming, 1942, The Oceans, Prentice-Hall.

Domogalla, B. P. 1935. Eleven Years of Chemical Treatment of the Madison Lakes: Its Effect on Fish and Fish Food. Trans. Amer. Fish. Soc. 65:115-120.

Doudoroff, P., and M. Katz. 1953. Critical Review of the Literature on the Toxicity of Industrial Wastes and Their Components to Fish. II. The Metals, as Salts. Sewage & Ind. Wastes 25:802-839.

Dragovich, A. 1961. Relative Abundance of Phytoplankton off Naples, Florida, and Associated Hydrographic Data, 1956-57. U.S. Fish Wildl. Serv., SSR-F 372, p. 41.

Dragovich, A., J. H. Finucane, and B. Z. May. 1961. Count of Red Tide Organisms, *Gymnodinium breve,* and Associated Oceanographic Data from Florida West Coast, 1957-59. U.S. Fish Wildl. Serv., SSR-F 369, 175 p.

Droop, M. R. 1958. Requirement for Thiamine Among Some Marine and Supra-Littoral Protista. J. Mar. Biol. Assn. U.K. 37:323-329.

Droop, M. R. 1973. Some thoughts on Nutrient Limitation in Algae. J. Phycol. 9:264-272.

Dugdale, R. C., and J. J. MacIsaac. 1971. A Computation Model for the Uptake of Nitrate in the Peru Upwelling Region. Inv. Pesq. 35:299-308.

Duursma, E. K., and W. Sevenhuysen. 1966. Note on Chelation and Solubility of Certain Metals in the Sea Water at Different pH Values. Netherlands J. Sea Res. 3:95-106.

Ellis, M. M. 1937. Detection and Measurement of Stream Pollution. Bull. Bur. Fish., No. 22, 48:365-437.

Eppley, R. W., and J. L. Coatsworth. 1968. Uptake of Nitrate and Nitrite by *Ditylum brightwellii* - Kinetics and Mechanisms. J. Phycol. 4: 151-156.

Eppley, R. W., J. N. Rogers, and J. J. McCarthy. 1969. Half-Saturation Constants for Uptake of Nitrate and Ammonium by Marine Phytoplankton. Limnol. Oceanogr. 14:912-919.

Eppley, R. W., and J. N. Rogers. 1970. Inorganic Nitrogen Assimilation of *Ditylum brightwellii*. A Marine Diatom. J. Phycol. 6:344-351.

Erickson, S. J., N. Lackie, and T. E. Maloney. 1970. A Screening Technique for Estimating Copper Toxicity to Estuarine Phytoplankton. J. Water Pollution Control Fed. 42:R270-R278.

Feinstein, A., R. Ceurvels, R. F. Hutton, and E. Snoek. 1955. Red Tide Outbreaks off the Florida West Coast. Mar. Lab., Univ. Miami, Report 55-15 to Fla. St. Bd. Conserv., 44 pp.

Feustel, I. C., and H. G. Byers. 1930. The Physical and Chemical Characteristics of Certain American Peat Profiles. U.S. Dept. Agri., Tech. Bull. 214, 27 pp.

Finucane, J. H., and A. Dragovich. 1959. Counts of Red Tide Organisms, *Gymnodinium breve,* and Associated Oceanographic Data from Florida West Coast, 1954-57. U.S. Fish Wildl. Serv., SSR-F 289, 220 pp.

Fisher, A. 1956. The Effects of Copper-Sulfate on Some Microorganisms in Fish Ponds. Bamidgeh, Bull. Fish Culture in Israel 8:21-26.

Fitzgerald, G. P., and W. B. Lyons. 1973. Organic Mercury Compounds in Coastal Waters. Nature 242:452.

Fogg, G. E., and D. F. Westlake. 1955. The Importance of Extracellular Products of Algae in Freshwater. Limnol. 12:219-232.

Freeberg, L. R. 1971. The Interrelationship of Light and Temperature on the Growth Rate of *Isochrysis galbana* Parke (Chrysophyta) in Continuous Culture. M.S. Thesis. Texas A&M Univ., College Station. 59 pp.

Frudenthal, H. D., and J. J. Lee. 1963. *Glenodinium halli* n. sp. and *Gyrodinium instriatum* n. sp., dinoflagellates from New York waters. J. Protozool. 10:182-189.

Galtsoff, P. S. 1948. Red Tide. Progress Report on the Investigation of the Cause of the Mortality of Fish Along the West Coast of Florida Conducted by the U. S. Fish and Wildlife Service and Cooperating Organizations. U.S. Fish Wildl. Serv., SSR 46, 44 pp.

Galtsoff, P. S. 1943. Copper Conent of Sea Water. Ecology 24:263-265.

Gates, J. E., and W. B. Wilson. 1960. The Toxicity of *Gonyaulax monilata* Howell to *Mugil cephalus*. Limnol. Oceanogr. 5:171-174.

Glooschenko, W. A. 1969. Accumulation of ^{203}Hg by the marine diatom *Chaetoceros costatum*. J. Phycol. 5:224-226.

Guillard, R. R. L., and J. H. Ryther. 1962. Studies of Marine Planktonic Diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Can. J. Micro. 8:229-239.

Gunter, G., R. H. Williams, C. C. Davis, and F.G.W. Smith. 1948. Catastrophic Mass Mortality of Marine Animals and Coincident Phytoplankton Bloom on the West Coast of Florida, November 1946 to August 1947. Ecol. Mono. 18:309-324.

Hale, F. E. 1950. The Use of Copper Sulfate in Control of Microscopic Organisms. 5th Ed., Phelps Dodge Refining Corp., New York, 44 pp.

Hammer, H. E. 1929. The Chemical Composition of Florida Everglades Peat Soils. Soil Science 28:1-14.

Harriss, R. C., D. B. White, and R. B. MacFarlane. 1970. Mercury Compounds Reduce Photosynthesis by Plankton. Science 170:736-737.

Harvey, H. W. 1939. Substances Controlling the Growth of a Diatom. J. Mar. Biol. Assn. U.K. 23:499-520.

Harvey, H. W. 1955. The Chemistry and Fertility of Sea Waters. Cambridge Univ. Press, 224 pp.

Hodgman, C. D. (ed.) 1938. Handbook of Chemistry and Physics, 30th Ed. Chemical Rubber Publ. Co., Cleveland, Ohio 2686 pp.

Hunter, W. R. 1950. The Poisoning of *Marinogammarvs marinus* by Cupric Sulfate and Mercuric Chloride. J. Exp. Biol. 25:113-124.

Hutner, S. H., L. Provasoli, A. Schatz, and C. P. Haskins. 1950. Some Approaches to the Study of the Role of Metals in the Metabolism of Microorganisms. Proc. Amer. Philos. Soc. 94:152-170.

Hutner, S. H., and L. Provasoli. 1951. The Phytoflagellates. In: Biochemistry and Physiology of Protozoa, vol. 1, A. Lwoff, ed. Academic Press, New York, pp. 27-128.

Jerlov, N. G. 1951. Optical Studies of Ocean Waters. In: Report of the Swedish Deep-Sea Expedition, vol. 3, A. Petterson, ed. Elanders Baktryekeri Aktiebolog, Goteborg.

Jerlov, N. G. 1954. Colour Filters to Simulate the Extinction of Daylight in the Sea. J. Cons. Perm. Int. Explor. Mer. 20:156-159.

Johnston, R. 1964. Sea Water, the Natural Medium of Phytoplankton. II. Trace Metals and Chelation, and General Discussion. J. Mar. Biol. Assn. U.K. 44:87-109.

Jones, J.R.E. 1938. The Relative Toxicity of Salt, Lead, Zinc and Copper to the Stickleback, *Gasterosteus aculeatus* L., and the Effects of Calcium on the Toxicity of Lead and Zinc Salt. J. Exp. Biol. 15:394-407.

Jones, J.R.E. 1939. The Relation Between the Electrolytic Solution Pressures of the Metals and Their Toxicity to the Stickleback, *Gasterosteus aculeatus* L. J. Exp. Biol. 16:425-437.

Kain, J. M., and G. E. Fogg. 1958. Studies on the Growth of Marine Phytoplankton: II. *Isochrysis galbana*, Parke. J. Mar. Biol. Assn. U.K. 37:781-788.

Kamp-Nielsen, L. 1971. The Effect of Deleterious Concentrations of Mercury on the Photosynthesis and Growth of *Chlorella pyrenoidosa*. Physiol. Plant. 24:556-561.

Ketchum, B. H., and A. C. Redfield. 1949. Some Physical and Chemical Characteristics of Algae Grown in Mass Culture. J. Cell. Comp. Physiol. 33:281-300.

King, G. S. 1950. Production of Red Tide in the Laboratory. Proc. Gulf Caribb. Fish. Inst., 2nd Ann. Sess., Miami Beach, pp. 107-109.

King, J. E. 1950. A Preliminary Report on the Plankton of the West Coast of Florida. Quart. J. Fla. Acad. Sci. 12:109-137.

Knauer, G. A., and J. H. Martin. 1972. Mercury in a Marine Pelagic Food Chain. Limnol. Oceanogr. 17:868-876.

Kuenzler, E. J., and B. H. Ketchum. 1962. Rate of Phosphorus Uptake by *Phaeodactylum tricornutum*. Biol. Bull. 123:134-145.

Long, E. B., and G. D. Cooke. 1971. A Quantitative Comparison of Pigment Extraction by Membrane and Glass-Fiber Filters. Limnol. Oceanogr. 16:990-992.

Lorenzen, C. J. 1966. A Method for the Continuous Measurement of *in vivo* Chlorophyll Concentration. Deep-Sea Res. 13:223-227.

Lwoff, A., and E. Lederer. 1935. Remarque sur "1' extrait de terre" envisage comme facteur de croissance pour les Hagelle's. Compte Rend. Hebromadairedes Seances et Memoires de la Societe de Biologie, Paris 119:971-973.

Lyon, T. L., and H. O. Buckman. 1943. The Nature and Proper Ties of Soils. The Macmillan Co., New York, 499 pp.

Malek, I. 1966. A Theoretical Analysis of Continuous Culture Systems. in: Theoretical and Methodological Basis of Continuous Culture of Microorganisms, I. Malek and Z. Fenel, eds. Academic Press, New York.

Mandelli, E. F. 1969. The Inhibitory Effects of Copper on Marine Phytoplankton. Cont. Mar. Sci. 14:47-57.

Martell, A. E., and M. Calvin. 1952. Chemistry of Metal Chelate Compounds. Prentice-Hall, New York, 613 pp.

Marvin, K. T., L. M. Lansford, and R. S. Wheeler. 1960. Effects of Copper Ore on the Ecology of a Lagoon. U.S. Fish Wildl. Serv., Fish. Bull. 184, 61:133-160.

Mason, B. 1952. Principles of Geochemistry. John Wiley & Sons, New York, 276 pp.

Matsudaira, C. 1942. On Inorganic Sulfide as a Growth-Promoting Ingredient for Diatom. Proc. Imperial Academy, Japan 14:55-64.

Mattern, C.F.T., F. S. Brackett, and B. J. Olson. 1957. Determination of Number and Size of Particles by Electrical Gating: Blood Cells. J. Appl. Physiol. 10:56-70.

Miller, M. A. 1946. Toxic Effects of Copper on Attachment and Growth of *Bugula neritina*. Biol. Bull. 90:122-140.

Monie, W. D. 1956. Algae Control with Copper Sulfate. Water & Sewage Works 103:392-397.

Moore, G. T., and K. F. Kellerman. 1904. A Method of Destroying or Preventing the Growth of Algae and Certain Pathogenic Bacteria in Water Supplies. Bur. Plant Ind., Bull. 64, 64 pp.

Moore, G. T., and K. F. Kellerman. 1905. Copper as an Algicide and Disinfectant in Water Supplies. Bur. Plant Ind., Bull. 76, 55 pp.

Morita, Y. 1950. Distribution of Copper and Zinc. IV. Copper and Zinc in Sea Water. J. Chem. Soc. Japan 71:246-248.

Niemi, A. 1972. Effect of Toxicants on Brackish-Water Phytoplankton Assimilation. Commen. Biol. 55:1-19.

Novick, A., and L. Sziland. 1950. Experiments with the Chemostat on Spontaneous Mutations of Bacteria. Proc. Nat. Acad. Sci. 36:708-719.

Nuzzi, R. 1972. Toxicity of Mercury to Phytoplankton. Nature, London 237:38-40.

O'Brien, J. W. 1974. The Dynamics of Nutrient Limitation of Phytoplankton Algae: A Model Reconsideration. Ecology 55:135-141.

Platt, T., and D. V. Subba Rao. 1978. Some Current Problems in Marine Phytoplankton Productivity. Fish. Res. Bd. Canada, Tech. Rept. 370, 70 pp.

Pop, L.J.J. 1936. The Influence of Hydrogen Sulfide on Growth and Metabolism of Green Algae, W. D. Meinema, Delft. (Doctoral thesis - In English with Dutch Summary), 147 pp.

Powell, E. O. 1955. Growth Rate and Generation Time of Bacterial, With Special Reference to Continuous Culture. J. Gen. Micro. 15:492-511.

Powers, E. B. 1920. Influence of Temperature on the Toxicity of Salts to Fishes. Ecology 1:95-112.

Prakash, A., and M. A. Rashid. 1968. Influence of Humic Substances on the Growth of Marine Phytoplankton: Dinoflagellates. Limnol. Oceanogr. 13:598.

Provasoli, L. 1956. Growth Factors in Unicellular Marine Algae. Perspective in Marine Biology, A. A. Buzzati-Traverso, ed. Scripps Inst. Oceanog., Univ. Calif., pp. 385-403.

Provasoli, L. 1958. Nutrition and Ecology of Protozoa and Algae. Ann. Rev. Micro. 12:279-308.

Provasoli, L., J.J.A. McLaughlin, and I. J. Pintner. 1954. Relative and Limiting Concentrations of Major Mineral Constituents for the Growth of Algal Flagellates. N.Y. Acad. Sci., Ser. II 16:412-417.

Provasoli, L., and I. J. Pintner. 1953. Ecological Implications of *in vitro* Nutritional Requirements of Algal Flagellates. Ann. N.Y. Acad. Sci. 56:839-851.

Provasoli, L., J.J.A. McLaughlin, and M.R. Droop. 1957. The Development of Artificial Media for Marine Algae. Archiv für Mikrobiologie, 25: 392-428.

Prytherch, H. F. 1939. The Role of Copper in the Setting, Metamorphosis, and Distribution of the American Oyster, *Ostrea (Crassostrea) virginica*. Ecol. Mono. 4:47-107.

Pyefinch, K. A., and J. C. Mott. 1948. The Sensitivity of Barnacles and Their Larvae to Copper and Mercury. J. Exp. Biol. 25:276-298.

Rankama, K., and Th. G. Sahama. 1950. Geochemistry. University of Chicago Press, Chicago, Ill., 912 pp.

Ray, S. M., and W. B. Wilson. 1957. Effect of Unialgal and Bacteria-Free Cultures of *Gymnodinium brevis (G. breve)* on fish. U.S. Fish Wildl. Serv., Fish. Bull. 123, 57:469-496.

Rhee, G-Y. 1973. A Continuous Culture Study of Phosphate Uptake, Growth Rate and Polyphosphate in *Scenedesmus* sp. J. Phycol. 9:495-506.

Rice, H. V., D. A. Leighty, and G. C. McLeod. 1973. The Effects of Some Trace Metals on Marine Phytoplankton. Critical Rev. Microbiol., vol. 3.

Richet, C. 1881. De la toxicite comparie das differents metaux. Compte Rendu Hebdomadaire des Seances de l'Academie des Sciences, Paris, 93:649-651. (In Doudoroff and Katz, 1953)

Riley, G. A. 1937. The Significance of the Mississippi River Drainage for Biological Conditions in the Northern Gulf of Mexico. J. Mar. Res. 1:60-74.

Rothstein, A. 1959. Cell Membrane as Site of Action of Heavy Metals. Proc. Fed. Amer. Soc. Exp. Biol. 18:1026-1038.

Rounsefell, G. A., and J. E. Evans. 1958. Large-scale Experimental Test of Copper Sulfate as a Control for the Florida Red Tide. U.S. Fish Wildl. Serv., SSR-F 270, 57 pp.

Rudolfs, W., and A. L. Zuber. 1953. Removal of Toxic Material by Sewage Sludge. Sewage & Ind. Wastes 25:142-154.

Ryther, J. H. 1955. Ecology of Autotrophic Marine Dinoflagellates with Reference to Red Water Conditions. In: The Luminescence of Biological Systems, F. H. Johnson, ed. Proc. Conference on Luminescence, 1954, sponsored by Committee on Photobiology, National Academy of Sciences, National Research Council. AAAS, pp. 387-414.

Ryther, J. H. 1956. Photosynthesis in the Ocean as a Function of Light Intensity. Limnol. Oceanogr. 1:61-70.

Saunders, G. W. 1957. Interrelations of Dissolved Organic Matter and Phytoplankton. Botan. Rev. 23:389-410.

Schreiner, O., and F. C. Shorey. 1909. The Isolation of Harmful Organic Substances from Soils. Bur. Soils, U.S. Dept. Agri., Bull. 53, 53 pp.

Shilo (Shelubsky), M., S. Sarig, M. Shilo and H. Zeer. 1954. Control of *Prymnesium parvum* in Fish Ponds with the Aid of Copper Sulfate. Bamidgeh, Bull. Fish Culture in Israel 6:99-102.

Slobodkin, L. B. 1953. A Possible Initial Condition for Red Tides on the Coast of Florida. J. Mar. Res. 12.

Slowey, J. F., L. M. Jeffrey, and D. W. Wood. 1967. Evidence for Organic Complexed Copper in Sea Water. Nature 214:377.

Spencer, C. P. 1952. On the Use of Antibiotics for Isolating Bacteria-Free Cultures of Marine Phytoplankton Organisms. J. Mar. Biol. Assn. U.K. 31:97-106.

Spencer, C. P. 1957. Utilization of Trace Elements by Marine Unicellular Algae. J. Gen. Micro. 16:282-285.

Starr, T. J., and M. E. Jones. 1957. The Effect of Copper on the Growth of Bacteria Isolated from Marine Environments. Limnol. Oceanogr. 2: 33-36.

Steemann-Nielsen, E., and S. Wium-Anderson. 1970. Copper Ions as Poison in the Sea and in Fresh Water. Mar. Biol. 6:93-97.

Strickland, J.D.H. 1965. Production of Organic Matter in the Primary Stages of the Marine Food Chain. In: Chemical Oceanography, vol. 1, J. P. Riley and G. Skirraw, eds. Academic Press, New York.

Strickland, J.D.H., and T.R. Parsons. A Practical Handbook of Seawater Analysis. Fish. Res. Bd. Canada, Bull. 167.

Sverdrup, H. U., M. W. Johnson, and R. H. Fleming. 1942. The Oceans, Their Physics, Chemistry and General Biology. Prentice-Hall, New York, 1087 pp.

Sweeney, B. M. 1954. *Gymnodinium splendens,* a Marine Dinoflagellate Requiring B_{12}. Amer. J. Botany 41:821-824.

Taylor, W. R. 1960. Some Results of Studies on the Uptake of Radioactive Waste Materials by Marine and Estuarine Phytoplankton Organisms Using Continuous Culture Techniques. Chesapeake Bay Inst., Johns Hopkins Univ., Tech. Rept. No. 21.

Thomas, A. 1915. Effects of Certain Metallic Salts Upon Fishes. Trans. Amer. Fish. Soc. 44:120-124.

Thomas, W. H., and A. N. Dodson. 1978. Effects of Phosphate Concentration on Cell Division Rates and Yield of a Tropical Oceanic Diatom. Biol. Bull. 134:199-208.

Tiffin, L. O., and J. C. Brown. 1959. Absorption of Iron from Iron Chelate by Sunflower Roots. Science 130:274-275.

Waksman, S. A., D. B. Johnson, and C. L. Carey. 1942. The Effect of Copper Upon the Development of Bacteria in Sea Water and the Isolation of Specific Bacteria. J. Mar. Res. 5:136-152.

Whipple, G. C. 1947. The Microscopy of Drinking Water. John Wiley & Sons, New York and Chapman and Hall, London. (Revised by G. M. Fair and M. C. Whipple), 586 pp.

Wilson, W. B. 1958. Compounds Toxic to Red Tide Organisms. U.S. Fish Wildl. Serv. Ann. Rept., Gulf Fisheries Investigations.

Wilson, W. B. 1972. Toxicity of Metals to Marine PHytoplankton Cultures. Comprehensive Prog. Rept., EPA Grant 18050 DUI and 18080 DUI.

Wilson, W. B., and A. Collier. 1955. Preliminary Notes on Culturing *Gymnodinium brevis* Davis. Science 121:394-395.

Wolff, G., S. Fallab, and H. Erlenmeyer. 1955. Metallic Ions and Biological Activity. XXXVIII. The Binding Capacity for Cupric Ions of Some Amino Acids and Peptides. Experientia 11:440-442.

Woods Hole Oceanographic Institution. 1952. Marine Fouling and Its Prevention. U.S. Naval Inst., Annapolis, Md., 388 pp.

Yentsch, C. S., and D. W. Menzel. 1963. A Method for the Determination of Phytoplankton Chlorophyll and Phaeophytin by Fluorescence. Deep-Sea Res. 10:221-231.

ZoBell, C. E., and D. Q. Anderson. 1936. Vertical Distribution of Bacteria in Marine Sediment. Amer. Assoc. Petroleum Geologists, Bulletin, 20:258-269.

The typists for this Report were Mrs. Margie Watson and Ms. Nora McKenna